Central Nervous System Drug Evaluation Using Positron Emission Tomography

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In conventional pharmacological research in the field of mental disorders, pharmacological effect and dose have been estimated by ethological approach and in vitro data of affinity to the site of action. In addition, the frequency of administration has been estimated from drug kinetics in blood. However, there is a problem regarding an objective index of drug effects in the living body. Furthermore, the possibility that the concentration of drug in blood does not necessarily reflect the drug kinetics in target organs has been pointed out. Positron emission tomography (PET) techniques have made progress for more than 20 years, and made it possible to measure the distribution and kinetics of small molecule components in living brain. In this article, we focused on rational drug dosing using receptor occupancy and proof-of-concept of drugs in the drug development process using PET.

KEY WORDS: Positron emission tomography; Occupancy; Dopamine D2 receptor; Serotonin transporter; Norepinephrine transporter; micro-PET.

INTRODUCTION

In conventional pharmacological research in the field of mental disorders, pharmacological effect and dose have been estimated by ethological approach and in vitro data of affinity to the site of action. In addition, the frequency of administration has been estimated from drug kinetics in blood. However, there is a problem regarding an objective index of drug effects in the living body. Furthermore, the possibility that the concentration of drug in blood does not necessarily reflect the drug kinetics in target organs has been pointed out. Positron emission tomography (PET) techniques have made progress for more than 20 years, and made it possible to measure the distribution and kinetics of small molecule components in living brain, PET neuroimaging including neurotransmitter imaging and enzyme activity imaging have contributed to drug evaluation by 1) rational drug dosing, 2) biodistribution of drug, 3) therapeutic rationale for drug utilization, and 4) mechanism of drug action.1 In this article, we focused on rational drug dosing using receptor occupancy and proof-of-concept of drugs in the drug development process using PET.

Dopamine D2 Receptor Occupancy by Antipsychotics

Dopamine dysregulation has been suspected for the pathophysiology of schizophrenia. The common pharmacological profile of antipsychotics that can alleviate positive symptoms has a dopamine D2 receptor blocking property.2 Farde and others succeeded in visualizing dopamine D2 receptors by using the selective, high-affinity dopamine D2 receptor antagonist 11C-labeled raclopride and PET, allowing estimation of dopamine D2 receptor bindings quantitatively in the human brain.3 By applying this technique, it was possible to evaluate the degree of dopamine D2 receptor inhibition of antipsychotics as a change of radioligand binding. Using a binding potential (BP) reflecting the receptor density at the specific binding site, occupancy was defined as the percentage reduction of BP and calculated as follows:

\[ \text{Occupancy(\%)} = \frac{\text{BP}_{\text{baseline}} - \text{BP}_{\text{drug}}}{\text{BP}_{\text{baseline}}} \times 100 \]

There are some reports concerning dopamine D2 receptor occupancy by antipsychotics in living human brain. A range of 70 to 89% was reported in an open study of 22
schiophrenic patients responding to treatment with conventional doses of classical neuroleptics. A double-blind PET study of schizophrenic patients suggested that dopamine D2 receptor occupancy was positively correlated with the percentage of reduction in total Brief Psychopathological Rating Scale (BPRS) score at the end of treatment compared to baseline, and the dopamine D2 receptor occupancy value required to induce 50% reduction of BPRS was about 70%. Another double-blind PET study reported a significant relationship between dopamine D2 receptor occupancy and improvement in Clinical Global Impressions Scale (CGI) rating, with over 65% dopamine D2 receptor occupancy showing a distinct clinical response. On the basis of these findings, the likelihood of clinical response increases as dopamine D2 receptor occupancy exceeds 70%, while the risks of extrapyramidal symptoms (EPSs) increase at occupancy higher than 80%.

Dose-finding of Antipsychotics Based on Dopamine D2 Receptor Occupancy

Appropriate dosages of various antipsychotics are now being decided based on measurements of dopamine D2 receptor occupancy. Previous studies reported that a dose range between 3 and 5 mg/day of risperidone was assumed to be optimal for supporting the clinical outcome. Moreover, the appropriate dosage of olanzapine has been reported to be 8-14 mg/day, also in good agreement with the clinical dose. In a phase II clinical trial in Japan, paliperidone ER at 6-9 mg/day provides an estimated level of dopamine D2 receptor occupancy between 70-80%. In Korea as well, dopamine D2 receptor occupancy by a novel antipsychotic, YKP1358, was measured using PET, and this will require further clinical study.

Thus, performing a dose-finding study using PET at the clinical trial stage has been considered one of the reasons for the fewer side effects of the so-called second-generation antipsychotics.

On the other hand, in terms of conventional antipsychotics, there have not been enough supporting data regarding clinical doses based on dopamine D2 receptor occupancy. Takano and others measured dopamine D2 receptor occupancy of two conventional benzamide antipsychotics, sulpiride and sultopride, using positron emission tomography, to investigate the rationale of their clinical doses. In that study, doses required for 70-80% occupancy were shown to be quite different: 1,010-1,730 mg for sulpiride but 20-35 mg for sultopride despite their similar registered clinical doses (300-1,200 mg). Sultopride has been reported to induce more EPSs than sulpiride, which can be attributed to the fact that the registered clinical doses of sultopride were approximately 10 times higher than the calculated optimal doses.

As evidence for the clinical doses of conventional antipsychotics has been limited, their re-evaluation based on dopamine D2 receptor occupancy can contribute to the es-
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Fig. 3. Time-course of D2 receptor occupancy by risperidone.17) Time-course of dopamine D2 receptor occupancy in the temporal cortex (●) and the plasma concentrations (○) after taking 4 mg risperidone. The sum of the plasma concentrations of risperidone and 9-OH-risperidone was used as the plasma concentration of risperidone. The T1/2 of plasma concentration (17.7 h) was shorter than that of dopamine D2 receptor occupancy (73.8 h).

establishment of rational antipsychotic therapy.

Although the concept of a “therapeutic window” between 70-80% of dopamine D2 receptor occupancy seems to apply for most antipsychotics, there may be a different optimal occupancy other than 70-80% depending on the characteristic of drugs such as aripiprazole, dopamine D2 receptor partial agonist, occupy more than 90% of striatal dopamine D2 receptor at clinically effective doses.14,15) Furthermore, optimal occupancy of low-affinity drugs such as clozapine and quetiapine have been inconclusive.16)

Pharmacokinetics at Specific Binding Site

Until now, drug disposition has been mainly evaluated by plasma kinetics. However, it is important to directly focus on the kinetic profile at the specific binding site, except for the drugs that have a site of action in blood. Therefore, the time-course of occupancy at the binding site of the drug reflects the drug kinetics at the specific binding site, and is an important index. Takano and others measured the time-course of dopamine D2 receptor occupancy in the temporal cortex as well as that of risperidone plasma concentration after its administration in chronically treated patients.17) The half-life of the plasma concentration (17.7 h) was shorter than that of dopamine D2 receptor occupancy (73.8 h) (Fig. 3). Furthermore, they reported that the estimated time-course of dopamine D2 receptor occupancy from the mean pharmacokinetics data and the in-vivo ED50 value fitted well with the data from consecutive PET scans. Thus, estimating the drug kinetics at the receptor site in this way can applicable to appropriate dose scheduling.

Regionality of dopamine D2 Receptor Occupancy

The concept of limbic and cortical selectivity of second-generation antipsychotics, i.e., higher dopamine D2 receptor occupancy in the cerebral cortices than in the striatum, has also been suggested to explain their clinical efficacy with few EPSs.18) Limbic and cortical selectivity was originally observed in dopamine D2 receptor occupancy by clozapine in patients with schizophrenia using [123I]epidepride.18) Limbic and cortical selectivity was also reported in other second-generation antipsychotics such as risperidone and olanzapine.19,20) However, in most studies concerning the regional selectivity of dopamine D2 receptor occupancy in patients with schizophrenia, baseline binding to receptors for the calculation of occupancy was based on binding of other healthy subjects, not the binding of the neuroleptic-naive state of the same patients. In addition, dopamine D2 receptor density is quite different between striatal and extrastriatal regions. Therefore, to elucidate the regional difference in dopamine D2 receptor occupancy by second-generation antipsychotics, Ito and others measured dopamine D2 receptor occupancy in the striatal and extrastriatal regions by different tracers with different affinity for receptors using the neuroleptic-naive state of the same subjects as the baseline. No obvious regional differences in dopamine D2 receptor occupancy by risperidone were observed.21) Moreover, dopamine D2 receptor occupancy in the extrastriatal region by olanzapine has also been reported to agree with occupancy in the striatum.9) By adapting an appropriate measurement in this way, it could be shown that the concept of limbic and cortical selectivity of risperidone and olanzapine was not observed.

Dopamine D2 Receptor Occupancy in the Pituitary

Hyperprolactinemia, one of the common side effects of antipsychotic drugs, is reported to be induced by blocking of dopamine D2 receptors in the pituitary. Although examination of the relation between dopamine D2 receptor occupancy and hyperprolactinemia has been attempted using PET,6,22,23) the outcomes have been inconclusive. Arakawa and others reported that the dopamine D2 receptor occupancy in the pituitary by 4 antipsychotic drugs was significantly correlated with the plasma concentration of prolactin, but no such correlation was found in the temporal cortex (Fig. 4).24) Furthermore, a character-
Significant positive correlation was observed between the plasma concentration of prolactin and dopamine D2 receptor occupancy in the pituitary by different doses of risperidone, olanzapine, haloperidol, and sulpiride (Y=0.41; X=4.0; p=.001).

Serotonin Transporter Occupancy by Antidepressant

Serotonin transporters (5-HTT) are located at pre-synaptic serotonergic neurons and have a key role in the regulation of serotonin concentration in the synapse. They are believed to be one of the major therapeutic targets of antidepressants. PET studies, using radioligands such as \([^{11}C]\text{McN}+5652\) and \([^{11}C]\text{DASB}\), have made it possible to measure the occupancy of 5-HTT by antidepressants in living human brain. 5-HTT occupancy was reported to be over 80% at clinical doses of selective serotonin reuptake inhibitors (SSRIs) during the treatment of depression.25,26 Suhara and others investigated the relationship between 5-HTT occupancy and the dose of the classic tricyclic antidepressant (TCA) clomipramine, and one of the SSRIs, fluvoxamine.26 In this study, even 10 mg of clomipramine showed approximately 80% 5-HTT occupancy, while step-wise increases of fluvoxamine in dosage demonstrated only a gradual increase (Fig. 5). In a dose-finding study of duloxetine, a part of phase I clinical trials in Japan, it was reported that 40mg or more was needed to attain 80% occupancy, and 60 mg of duloxetine could maintain a high level of 5-HTT occupancy with a once-a-day administration schedule.27

However, a distinct threshold in 5-HTT occupancy for achieving an antidepressant effect without side effects, comparable to dopamine D2 receptor occupancy by antipsychotics, has not yet been demonstrated. Additionally, clomipramine, its metabolite desmethyl-clomipramine and duloxetine have affinity to norepinephrine transporter (NET) as well as 5-HTT. Although evaluation of NET occupancy is required, suitable radioligands for NET were not developed at the time these studies were carried out.

Norepinephrine Transporter Occupancy by Antidepressant

Norepinephrine, one of the monoamine neurotransmitters in the central nervous system, has been reported to be related to several functions such as memory, cognition, consciousness, emotion, etc. NET is responsible for the reuptake of norepinephrine into pre-synaptic nerves and is another main target of antidepressants such as serotonin-norepinephrine reuptake inhibitors (SNRIs) and TCAs. SSRIs are widely considered as the first choice of treatment for depression. However, it is known that about one-third of the patients with major depression do not respond to SSRIs.28,29 Recent studies have suggested that the treatment of depression with newer antidepressants that simultaneously enhance both serotonergic and noradrenergic neurotransmissions can be expected to result in higher response and remission rates compared to SSRIs.30,31 Therefore, examining NET occupancy by TCAs and SSRIs might provide a new therapeutic indication other than 5-HTT occupancy.

As mentioned above, several studies have been reported regarding 5-HTT occupancy by antidepressants. However, NET occupancy by antidepressants in human brain has not been reported because of a lack of suitable radioligands for NET. (S,S)-\([^{18}F]\text{FMeNER-D2}\) was recently developed as a radioligand for the measurement of NET binding with PET,32 allowing estimation of NET bindings quantitatively in human brain.33,34 Furthermore, NET occupancy by nortriptyline, corresponding to the administration dose and plasma concentration of nortriptyline, was observed in human brain using PET with
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Fig. 5. Serotonin transporter occupancy by SSRI fluvoxamine in rat and human brain.45) (left side) PET imaging of $[11C]$DASB distribution in rat and human brain before and after oral administration of fluvoxamine. (right side) Relationship between plasma concentration of fluvoxamine and 5-HTT occupancy in rat and human brain. The plasma concentration of fluvoxamine needed for 50% occupancy (EC$_{50}$=6.1 ng/ml) was almost equivalent to the value determined in human studies (EC$_{50}$=4.6 ng/ml).

(S,S)-$[18F]$FMeNER-D$_2$.36) Further NET occupancy studies in humans will be needed to evaluate the relation with the clinical effects of antidepressants.

Biomarkers for the Proof-of-Concept of Drugs and New Treatments

The most utilized imaging biomarker for rational drug dosing is the receptor or transporter occupancy by drugs acting on those sites as blockers. However, in psychiatric disorders, reliable diagnostic biomarkers are still awaited. Nonetheless, in the case of Alzheimer disease (AD), distinctive pathological changes such as deposition of $\beta$-amyloid protein (A $\beta$) and neurofibrillary tangles (NFT) have been identified. Quantification of A $\beta$ in living human brain is reported to represent an important diagnostic biomarker for AD. Several amyloid ligands, such as $[11C]$PIB, $[11C]$BF227, $[11C]$AZD-2184 and $[18F]$AV-45, have been developed for PET imaging.37-41)

Furthermore, Rinne and others reported that cortical $[11C]$PIB retention was reduced in the AD patients group received the treatment with bapineuzumab, a humanised anti-$\mathrm{A}\beta$ monoclonal antibody, compared with both baseline and the placebo group.42) Measurement of brain $\mathrm{A}\beta$ deposition by PET may be useful not only for early diagnosis of AD but also for therapeutic monitoring of the effects of disease-modifying agents such as anti-$\mathrm{A}\beta$ monoclonal antibody, secretase inhibitors or modifiers, metal-protein attenuating compounds, antioxidants and so on. Despite the distinctive biomarker and several promising therapeutic agents, current treatment of AD is limited to
Moreover, imaging microglia, which are observed in the encephalitis. [18F]FE-DAA1106 is an effective radio-

brain immune system, and their overactivation causes activation is responsible for the degree of activity of the vicinity of neuritic plaques, is also important because their was shown to be correlated with the doses and plasma con-
duced after vaccination measured by microPET system.40) In that study, a reduction of [11C]DASB binding to 5-HTT relation to radiological effects. In-vivo imaging with mi-
trials for new pharmaceuticals have various restrictions in development of new pharmaceuticals. However, clinical using amyloid precursor protein (APP) transgenic (Tg) mice, [ 11C]PIB binding was re-
tor the treatment is an issue to be settled before entering into any clinical trial. Using amyloid precursor protein (APP) transgenic (Tg) mice, [11C]PIB binding was re-
duced after vaccination measured by microPET system.40) Moreover, imaging microgla, which are observed in the vicinity of neuritic plaques, is also important because their activation is responsible for the degree of activity of the brain immune system, and their overactivation causes encephalitis. [18F]FE-DAA1106 is an effective radioligand for peripheral benzodiazepine receptor, and is regarded as an effective biomarker for activated microgla. Using two different ligands for amyloid deposition and microglial activation, a clear relationship between amyloid reduction and the activation of microgla was revealed in vaccinated Tg mice.44) This result suggests the usefulness of preclinical evaluation of emerging diagnostic and ther-
apeutic approaches for AD.45)

Sajjo and others investigated the occupancies of 5-HTT with rats treated with varying doses of fluvoxamine and a new compound, (2S)-1-[4-(3,4-dichlorophenyl) piperidin-1-yl]-3-[2-(5-methyl-1,3,4-oxadiazol-2-yl)benzo[b]fur-
an-4-yl]oxy]propan-2-ol monohydrochloride (Wf-516).45) In that study, a reduction of [11C]DASB binding to 5-HTT was shown to be correlated with the doses and plasma con-
centrations of fluvoxamine and Wf-516. The estimated ED50 value of Wf-516 for [11C]DASB binding was re-
sulted in 3.1 mg/kg (p.o.), which was 5 times lower than one of fluvoxamine as 15.2 mg/kg (p.o.). This ED50 ratio of Wf-516 to fluvoxamine was fairly close to previous ex vivo [3H]citaropram autoradiographic study (Wf-516 vs. fluvoxamine: 1.1 mg/kg vs. 4.5 mg/kg).46) Moreover, the plasma concentration of fluvoxamine needed for 50% occupancy of central 5-HTT was 6.1 ng/ml, similar to the value reported in human studies as 4.6 ng/ml (Fig. 5).47) These results suggest preclinical animal PET studies on candidate agents enabling the estimation of the sensitivity and efficacy of pharmacological agents in humans. However, there are several limitations in current small animal PET studies. The spatial resolution of those PET systems is limited to ~1.5 mm, which is not sufficient for analyzing small brain structures,48) and the anesthesia used for the fixation of the animal may influence the binding imaging agents.49,50)

**CONCLUSION**

In this article, the occupancy by antipsychotics and anti-depressants using PET was reviewed. Techniques using PET have made it possible to evaluate how each drug acts at the specific binding site, and provide important indices for evaluating clinical drug efficacy and side effects. Therefore, it is considered that their roles will continue to increase in the psychiatry domain, where there has perhaps been a shortage of objective indices.

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