Review
Clinical Psychopharmacology and Neuroscience 2011;9(1):9-16
pISSN 1738-1088 / eISSN 2093-4327

Central Nervous System Drug Evaluation Using Positron Emission Tomography

Mizuho Sekine[1,2], Jun Maeda[1], Hitoshi Shimada[1], Tsuyoshi Nogami[1,2], Ryosuke Arakawa[1,2], Harumasa Takano[1], Makoto Higuchi[1], Hiroshi Ito[1], Yoshiro Okubo[2], Tetsuya Suhara[1]

[1]Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, [2]Department of Neuropsychiatry, Nippon Medical School, Tokyo, Japan

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 D_2 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 neuroreceptor 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.

Received: October 31, 2010 / **Revised:** January 18, 2011
Accepted: January 19, 2011
Address for correspondence: Mizuho Sekine, MD, PhD
Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba, 263-8555, Japan
Tel: +81-43-206-3251, Fax: +81-43-253-0396
E-mail: sekine@nirs.go.jp

Dopamine D_2 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 D_2 receptor blocking property.[2)] Farde and others succeeded in visualizing dopamine D_2 receptors by using the selective, high-affinity dopamine D_2 receptor antagonist ^{11}C-labeled raclopride and PET, allowing estimation of dopamine D_2 receptor bindings quantitatively in the human brain.[3)] By applying this technique, it was possible to evaluate the degree of dopamine D_2 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:

$$Occupancy(\%) = \frac{BP_{baseline} - BP_{drug}}{BP_{baseline}} \times 100$$

There are some reports concerning dopamine D_2 receptor occupancy by antipsychotics in living human brain. A range of 70 to 89% was reportedin an open study of 22

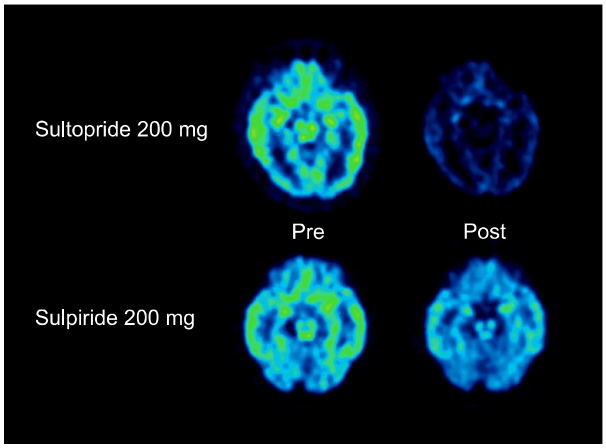

Fig. 1. Typical summated PET images before and after administration of 200mg of Sultopride and Sulpiride.[13] (upper stand) Pre is typical summated PET image before sultopride administration. Post is typical summated PET image at the possible peak time of plasma concentration of the sultopride, 2 hr after single administration of 200 mg of sultopride. (lower stand) Pre is typical summated PET image before sulpiride administration. Post is typical summated PET image at the possible peak time of plasma concentration of the sulpiride, 3 hr after single administration of 200 mg of sulpiride. The absolute decline in dopamine D_2 binding is significantly greater after administration of sultpride than sulpiride. PET, positron emission tomography.

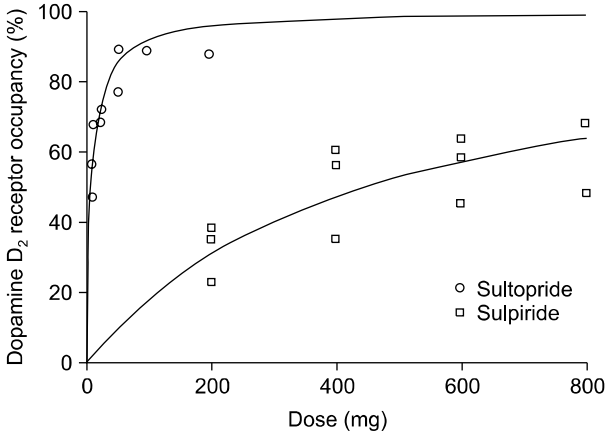

Fig. 2. Relationship between dopamine D_2 receptor occupancy and doses of sulpiride and sultopride.[13] Mean dopamine D_2 receptor occupancy of three regions (prefrontal cortex, temporal cortex, and thalamus) was shown as dopamine D_2 receptor occupancy. Open squares indicate sulpiride, and open circles indicate sultopride. The dotted regression curve was fitted to the sulpiride data, and the solid regression curve was fitted to the sultopride data.

schizophrenic patients responding to treatment with conventional doses of classical neuroleptics.[4] A double-blind PET study of schizophrenic patients suggested that dopamine D_2 receptor occupancywas 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 D_2 receptor occupancy valuerequired to induce 50% reduction of BPRS was about 70%.[5] Another double-blind PET study reported a significant relationship between dopamine D_2 receptor occupancy and improvement in Clinical Global Impressions Scale (CGI) rating, with over 65% dopamine D_2 receptor occupancy showing a distinct clinical response.[6] On the basis of these findings, the likelihood of clinical response increases as dopamine D_2 receptor occupancy exceeds 70%, while the risks of extrapyramidal symptoms (EPSs) increase at occupancy higher than 80%.

Dose-finding of Antipsychotics Based on Dopamine D_2 Receptor Occupancy

Appropriate dosages of various antipsychotics are now being decidedbased on measurements of dopamine D_2 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.[7,8] Moreover, the appropriate dosage of olanzapine has been reported to be 8-14 mg/day, also in good agreement with the clinical dose.[9,10] In a phase II clinical trial in Japan, paliperidone ER at 6-9mg/day provides an estimated level of dopamine D_2 receptor occupancy between 70-80% .[11] In Korea as well, dopamine D_2 receptor occupancy by a novel antipsychotic, YKP1358, was measured using PET,[12] 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 D_2 receptor occupancy. Takano and others measured dopamine D_2 receptor occupancy of two conventional benzamide antipsychotics, sulpiride and sultopride, using positron emission tomography, to investigate the rationale of their clinical doses.[13] 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) (Fig. 1, 2). 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 D_2 receptor occupancy can contribute to the es-

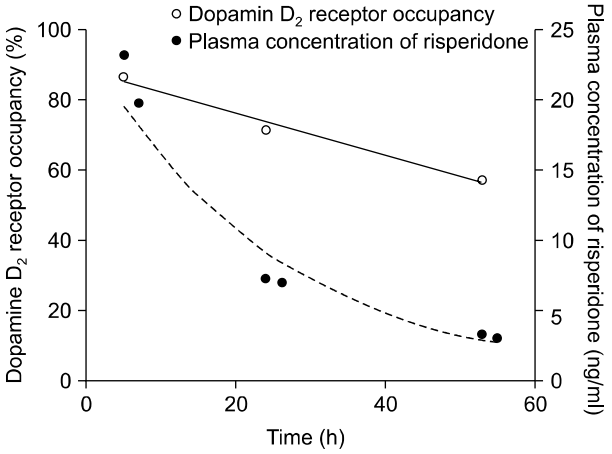

Fig. 3. Time-course of D_2 receptor occupancy by risperidone.[17] Time-course of dopamine D_2 receptor occupancy in the temporal cortex (●) and the plasma concentrations (○) after taking 4 mg risperidone. The sum of the plasma concentrations of risperidone and 9-OH-risperidone was used as the plasma concentration of risperidone. The $T_1/_2$ of plasma concentration (17.7 h) was shorter than that of dopamine D_2 receptor occupancy (73.8 h).

tablishment of rational antipsychotic therapy.

Although the concept of a "therapeutic window" between 70-80% of dopamine D_2 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 D_2 receptor partial agonist, occupy more than 90% of striatal dopamine D_2 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 D_2 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 D_2 receptor occupancy (73.8 h) (Fig. 3). Furthermore, they reported that the estimated time-course of dopamine D_2 receptor occupancy from the mean pharmacokinetics data and the in-vivo ED_{50} 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 D_2 Receptor Occupancy

The concept of limbic and cortical selectivity of second-generation antipsychotics, i.e., higher dopamine D_2 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 D_2 receptor occupancy by clozapine in patients with schizophrenia using [^{123}I]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 D_2 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 D_2 receptor density is quite different between striatal and extrastriatal regions. Therefore, to elucidate the regional difference in dopamine D_2 receptor occupancy by second-generation antipsychotics, Ito and others measured dopamine D_2 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 D_2 receptor occupancy by risperidone were observed.[21] Moreover, dopamine D_2 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 D_2 Receptor Occupancy in the Pituitary

Hyperprolactinemia, one of the common side effects of antipsychotic drugs, is reported to be induced by blocking of dopamine D_2 receptors in the pituitary. Although examination of the relation between dopamine D_2 receptor occupancy and hyperprolactinemia has been attempted using PET,[6,22,23] the outcomes have been inconclusive. Arakawa and others reported that the dopamine D_2 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-

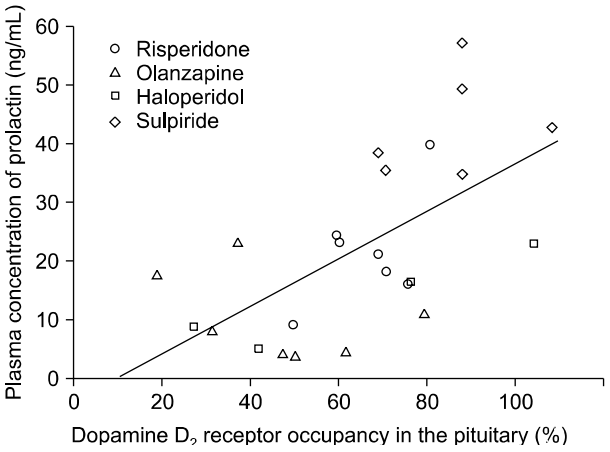

Fig. 4. Relation between plasma concentration of prolactin and dopamine D_2 receptor occupancy in the pituitary.[24] Significant positive correlation was observed between the plasma concentration of prolactin and dopamine D_2 receptor occupancy in the pituitary by different doses of risperidone, olanzapine, haloperidol, and sulpiride (Y=0.41; X-4.0; p=.001).

istic tendency of each drug was observed in dopamine D_2 receptoroccupancy in the pituitary and the temporal cortex. Sulpiride, known to be prone to hyperprolactinemia, blocked dopamine D_2 receptors in the pituitary more preferentially than in the temporal cortex, whereas olanzapine showed relatively less occupied dopamine D_2 receptors in the pituitary. This is due to the fact that the pituitary exists outside the blood-brain barrier. The magnitude of hyperprolactinemia of various antipsychotics can be predicted by the permeability of each antipsychotic into the brain. Thus, especially in the area of new drug development, the permeability of each antipsychotic drug might prove to be useful for the early evaluation of the risk of hyperprolactinemia.

Serotonin Transporter Occupancy by Antidepressant

Serotonin transporters (5-HTT) are located at presynaptic 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]McN(+)5652 and [^{11}C]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 D_2 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 NETwere 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 norepinephrinergic 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]FMeNER-D_2 was recently developed as a radioligand for the measurement of NET binding with PET,[32] allowing estimation of NET bindings quantitatively in human brain.[33-35] Furthermore, NET occupancy by nortriptyline, corresponding to the administration dose and plasma concentration of nortriptyline, was observed in human brain using PET with

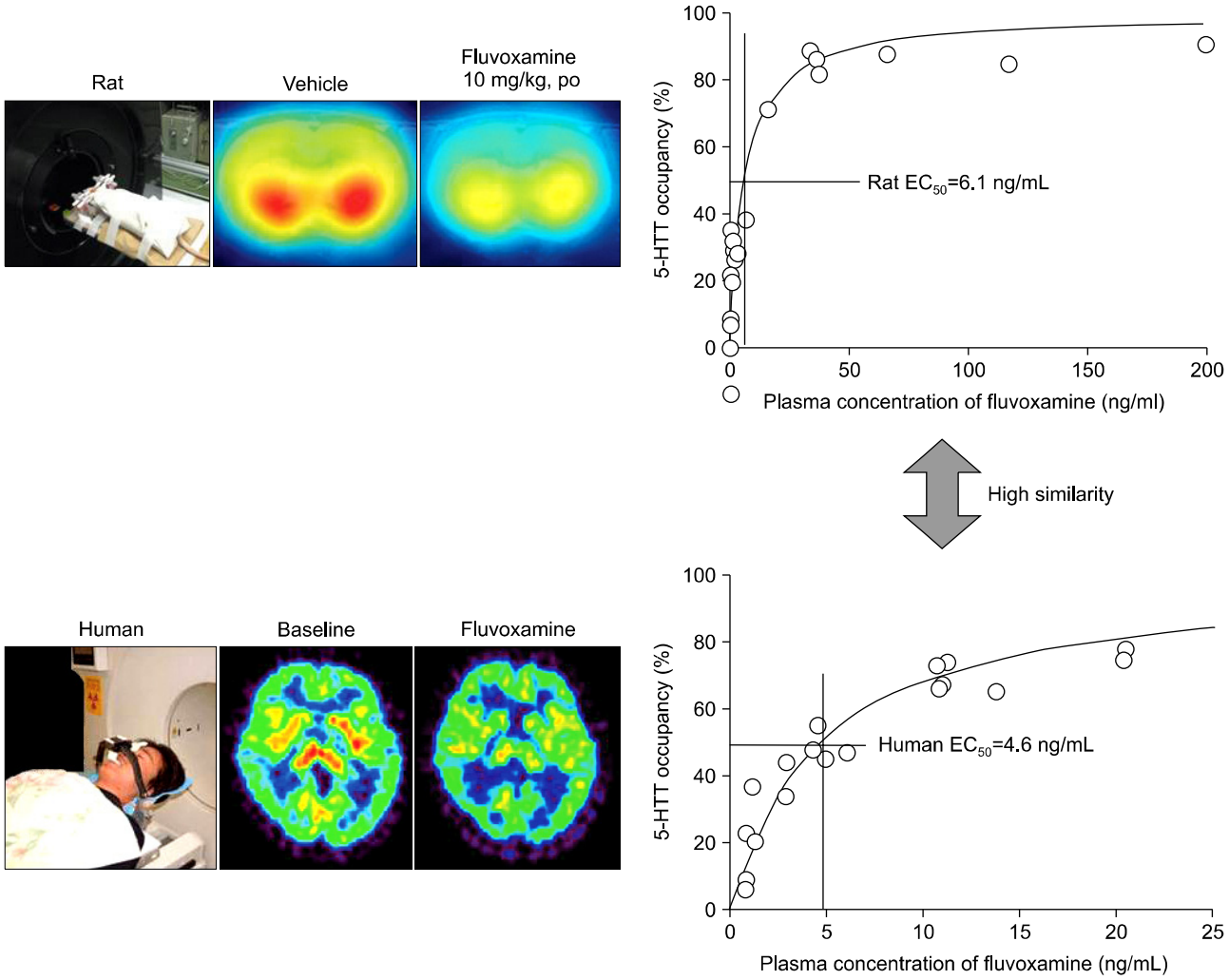

Fig. 5. Serotonin transporter occupancy by SSRI fluvoxamine in rat[45] and human brain.[47] (left side) PET imaging of ([11]C)DASB distribution in rat and human brain before and after oral administration of fluvoxamine. (right side) Relationship between plasma concentration of fluvoxamine and 5-HTT occupancy 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)-[^{18}F]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 β-amyloid protein (Aβ) and neurofibrillary tangles (NFT) have been identified. Quantification of Aβ in living human brain is reported to represent an important diagnostic biomarker for AD. Several amyloid ligands, such as [^{11}C]PIB, [^{11}C]BF227, [^{11}C]AZD-2184 and [^{18}F]AV-45, have been developed for PET imaging.[37-41]

Furthermore, Rinneand others reported that cortical [^{11}C]PIB retention was reduced in the AD patients group received the treatment with bapineuzumab, a humanised anti-Aβ monoclonal antibody, compared with both baseline and the placebo group.[42] Measurement of brain Aβ 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-Aβ 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

the enhancement of acetylcholinergic neurotransmission. This treatment is based on the pathology of a reduction in acetylcholinergic neurotransmission, thought to be responsible for several symptoms including memory impairment. Although an acetylcholinesterase inhibitor such as donepezil hydrochloride is assumed to increase the synaptic acetylcholine level, direct evidence of the mechanism of its action in the living human brain was needed. Using PET and radiolabeled acetylcholine analogue [^{11}C]N-methyl-4-piperidyl acetate (MP4A), also a substrate of acetylcholinesterase, and 5 mg of donepezil hydrochloride, acetylcholinesterase activity was reported to be reduced by 34.6% in AD brain.[43)]

Translational Study from Animal to Human

Measuring occupancy using PET is indispensable in the development of new pharmaceuticals. However, clinical trials for new pharmaceuticals have various restrictions in relation to radiological effects. In-vivo imaging with micro-PET of small animals is a more convenient means, and it is significant for preclinical drug development with the potential of simulating human models.

New concepts of treatment of AD are concerned with directly targeting amyloid in the brain. Vaccination therapy of Aβ is thought to be a candidate, but how to monitor the treatment is an issue to be settled before entering into any clinical trial. Using amyloid precursor protein (APP) transgenic (Tg) mice, [^{11}C]PIB binding was reduced after vaccination measured by microPET system.[40)] Moreover, imaging microglia, 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. [^{18}F]FE-DAA1106 is an effective radioligand for peripheral benzodiazepine receptor, and is regarded as an effective biomarker for activated microglia. Using two different ligands for amyloid deposition and microgial activation, a clear relationship between amyloid reduction and the activation of microglia was revealed in vaccinated Tg mice.[44)] This result suggests the usefulness of preclinical evaluation of emerging diagnostic and therapeutic approaches for AD.

Saijo 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] furan-4-yloxy]propan-2-ol monohydrochloride (Wf-516).[45)] In that study, a reduction of [^{11}C]DASB binding to 5-HTT was shown to be correlated with the doses and plasma concentrations of fluvoxamine and Wf-516. The estimated ED_{50} value of Wf-516 for [^{11}C]DASB binding was resulted in 3.1 mg/kg (p.o), which was 5 times lower than one of fluvoxamine as 15.2 mg/kg (p.o). This ED_{50} 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 6.1ng/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 antidepressants 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.

REFERENCES

1. Wong DF, Potter WZ, Brasic JR. *Proof of concept: functional models for drug development in humans. In: Davis KL, ed. Neuropsychopharmacology: The Fifth Generation of Progress: Lippincott Williams & Wilkins; 2002. p.457-473.*
2. Seeman P, Lee T. *Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 1975;188:1217-1219.*
3. Farde L, Hall H, Ehrin E, Sedvall G. *Quantitative analysis of D_2 dopamine receptor binding in the living human brain by PET. Science 1986;231:258-261.*
4. Farde L, Nordström AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. *Positron emission tomographic analysis of central D_1 and D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch Gen Psychiatry 1992; 49:538-544.*
5. Nordström AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C, *et al. Central D_2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biol Psychiatry 1993;33: 227-235.*

6. Kapur S, Zipursky R, Jones C, Remington G, Houle S. *Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 2000;157:514-520.*
7. Nyberg S, Eriksson B, Oxenstierna G, Halldin C, Farde L. *Suggested minimal effective dose of risperidone based on PET-measured D_2 and 5-HT2A receptor occupancy in schizophrenic patients. Am J Psychiatry 1999;156:869-875.*
8. Yasuno F, Suhara T, Okubo Y, Sudo Y, Inoue M, Ichimiya T, *et al. Dose relationship of limbic-cortical D_2-dopamine receptor occupancy with risperidone. Psychopharmacology (Berl) 2001;154:112-114.*
9. Arakawa R, Ito H, Okumura M, Takano A, Takahashi H, Takano H, *et al. Extrastriatal dopamine D(2) receptor occupancy in olanzapine-treated patients with schizophrenia. Eur Arch Psychiatry Clin Neurosci 2010;260:345-350.*
10. Kapur S, Zipursky RB, Remington G, Jones C, DaSilva J, Wilson AA, *et al. 5-HT2 and D_2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. Am J Psychiatry 1998;155:921-928.*
11. Arakawa R, Ito H, Takano A, Takahashi H, Morimoto T, Sassa T, *et al. Dose-finding study of paliperidone ER based on striatal and extrastriatal dopamine D_2 receptor occupancy in patients with schizophrenia. Psychopharmacology (Berl) 2008;197:229-235.*
12. Lim KS, Kwon JS, Jang IJ, Jeong JM, Lee JS, Kim HW, *et al. Modeling of brain D_2 receptor occupancy-plasma concentration relationships with a novel antipsychotic, YKP1358, using serial PET scans in healthy volunteers. Clin Pharmacol Ther 2007;81:252-258.*
13. Takano A, Suhara T, Yasuno F, Suzuki K, Takahashi H, Morimoto T, *et al. The antipsychotic sultopride is overdosed--a PET study of drug-induced receptor occupancy in comparison with sulpiride. Int J Neuropsychopharmacol 2006;9:539-545.*
14. Yokoi F, Grunder G, Biziere K, Stephane M, Dogan AS, Dannals RF, *et al. Dopamine D-2 and D-3 receptor occupancy in normal humans treated with the antipsychotic drug aripiprazole (OPC 14597): a study using positron emission tomography and [C-11]raclopride. Neuropsychopharmacology 2002;27:248-259.*
15. Grunder G, Carlsson A, Wong DF. *Mechanism of new antipsychotic medications: occupancy is not just antagonism. Arch Gen Psychiatry 2003;60:974-977.*
16. Gründer G, Landvogt C, Vernaleken I, Buchholz HG, Ondracek J, Siessmeier T, *et al. The striatal and extrastriatal D_2/D_3 receptor-binding profile of clozapine in patients with schizophrenia. Neuropsychopharmacology 2006;31:1027-1035.*
17. Takano A, Suhara T, Ikoma Y, Yasuno F, Maeda J, Ichimiya T, *et al. Estimation of the time-course of dopamine D_2 receptor occupancy in living human brain from plasma pharmacokinetics of antipsychotics. Int J Neuropsychopharmacol 2004;7:19-26.*
18. Pilowsky LS, Mulligan RS, Acton PD, Ell PJ, Costa DC, Kerwin RW. *Limbic selectivity of clozapine. Lancet 1997;350:490-491.*
19. Bressan RA, Erlandsson K, Jones HM, Mulligan RS, Ell PJ, Pilowsky LS. *Optimizing limbic selective D_2/D_3 receptor occupancy by risperidone: a [123I]-epidepride SPET study. J Clin Psychopharmacol 2003;23:5-14.*
20. Xiberas X, Martinot JL, Mallet L, Artiges E, Loc'H C, Mazière B, *et al. Extrastriatal and striatal D(2) dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. Br J Psychiatry 2001;179:503-508.*
21. Ito H, Arakawa R, Takahashi H, Takano H, Okumura M, Otsuka T, *et al. No regional difference in dopamine D_2 receptor occupancy by the second-generation antipsychotic drug risperidone in humans: a positron emission tomography study. Int J Neuropsychopharmacol 2009;12:667-675.*
22. Nordström AL, Farde L. *Plasma prolactin and central D_2 receptor occupancy in antipsychotic drug-treated patients. J Clin Psychopharmacol 1998;18:305-310.*
23. Bressan RA, Erlandsson K, Spencer EP, Ell PJ, Pilowsky LS. *Prolactinemia is uncoupled from central D_2/D_3 dopamine receptor occupancy in amisulpride treated patients. Psychopharmacology (Berl) 2004;175:367-373.*
24. Arakawa R, Okumura M, Ito H, Takano A, Takahashi H, Takano H, *et al. PET measurement of dopamine D_2 receptor occupancy in the pituitary and cerebral cortex: relation to antipsychotic-induced hyperprolactinemia. J Clin Psychiatry 2010;71:1131-1137.*
25. Meyer JH, Wilson AA, Sagrati S, Hussey D, Carella A, Potter WZ, *et al. Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study. Am J Psychiatry 2004;161:826-835.*
26. Suhara T, Takano A, Sudo Y, Ichimiya T, Inoue M, Yasuno F, *et al. High levels of serotonin transporter occupancy with low-dose clomipramine in comparative occupancy study with fluvoxamine using positron emission tomography. Arch Gen Psychiatry 2003;60:386-391.*
27. Takano A, Suzuki K, Kosaka J, Ota M, Nozaki S, Ikoma Y, *et al. A dose-finding study of duloxetine based on serotonin transporter occupancy. Psychopharmacology (Berl) 2006;185:395-399.*
28. Martin AJ, Tebbs VM, Ashford JJ. *Affective disorders in general practice. Treatment of 6000 patients with fluvoxamine. Pharmatherapeutica 1987;5:40-49.*
29. Stokes PE. *Fluoxetine: a five-year review. Clin Ther 1993;15:216-243.*
30. Papakostas GI, Thase ME, Fava M, Nelson JC, Shelton RC. *Are antidepressant drugs that combine serotonergic and noradrenergic mechanisms of action more effective than the selective serotonin reuptake inhibitors in treating major depressive disorder? A meta-analysis of studies of newer agents. Biol Psychiatry 2007;62:1217-1227.*
31. Thase ME, Pritchett YL, Ossanna MJ, Swindle RW, Xu J, Detke MJ. *Efficacy of duloxetine and selective serotonin reuptake inhibitors: comparisons as assessed by remission rates in patients with major depressive disorder. J Clin Psychopharmacol 2007;27:672-676.*
32. Schou M, Halldin C, Sóvágó J, Pike VW, Hall H, Gulyás B, *et al. PET evaluation of novel radiofluorinated reboxetine analogs as norepinephrine transporter probes in the monkey brain. Synapse 2004;53:57-67.*
33. Arakawa R, Okumura M, Ito H, Seki C, Takahashi H, Takano H, *et al. Quantitative analysis of norepinephrine transporter in the human brain using PET with (S,S)-18F-FMeNER-D_2. J Nucl Med 2008;49:1270-1276.*
34. Takano A, Gulyás B, Varrone A, Karlsson P, Schou M, Airaksinen AJ, *et al. Imaging the norepinephrine transporter with positron emission tomography: initial human studies with (S,S)-[18F]FMeNER-D_2. Eur J Nucl Med Mol Imaging 2008;35:153-157.*
35. Takano A, Varrone A, Gulyás B, Karlsson P, Tauscher J, Halldin C. *Mapping of the norepinephrine transporter in the human brain using PET with (S,S)-[18F]FMeNER-D_2.*

Neuroimage 2008;42:474-482.
36. Sekine M, Arakawa R, Ito H, Okumura M, Sasaki T, Takahashi H, *et al. Norepinephrine transporter occupancy by antidepressant in human brain using positron emission tomography with (S,S)-[18F]FMeNER-D$_2$. Psychopharmacology (Berl) 2010;210:331-336.*
37. Kudo Y. *Development of amyloid imaging PET probes for an early diagnosis of Alzheimer's disease. Minim Invasive Ther Allied Technol 2006;15:209-213.*
38. Kudo Y, Okamura N, Furumoto S, Tashiro M, Furukawa K, Maruyama M, *et al. 2-(2-[2-Dimethylaminothiazol-5-yl]ethenyl)-6- (2-[fluoro]ethoxy)benzoxazole: a novel PET agent for in vivo detection of dense amyloid plaques in Alzheimer's disease patients. J Nucl Med 2007;48:553-561.*
39. Nyberg S, Jönhagen ME, Cselényi Z, Halldin C, Julin P, Olsson H, *et al. Detection of amyloid in Alzheimer's disease with positron emission tomography using [11C]AZD$_2$184. Eur J Nucl Med Mol Imaging 2009;36:1859-1863.*
40. Choi SR, Golding G, Zhuang Z, Zhang W, Lim N, Hefti F, *et al. Preclinical properties of 18F-AV-45: a PET agent for Abeta plaques in the brain. J Nucl Med 2009;50: 1887-1894.*
41. Wong DF, Rosenberg PB, Zhou Y, Kumar A, Raymont V, Ravert HT, *et al. In vivo imaging of amyloid deposition in Alzheimer disease using the radioligand 18F-AV-45 (florbetapir [corrected] F 18). J Nucl Med 2010;51:913-920.*
42. Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, *et al. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. Lancet Neurol 2010;9:363-372.*
43. Ota T, Shinotoh H, Fukushi K, Kikuchi T, Sato K, Tanaka N, *et al. Estimation of plasma IC50 of donepezil for cerebral acetylcholinesterase inhibition in patients with Alzheimer disease using positron emission tomography. Clin Neuropharmacol 2010;33:74-78.*
44. Maeda J, Ji B, Irie T, Tomiyama T, Maruyama M, Okauchi T, *et al. Longitudinal, quantitative assessment of amyloid, neuroinflammation, and anti-amyloid treatment in a living mouse model of Alzheimer's disease enabled by positron emission tomography. J Neurosci 2007;27:10957-10968.*
45. Saijo T, Maeda J, Okauchi T, Maeda J, Morio Y, Kuwahara Y, *et al. Utility of small-animal positron emission tomographic imaging of rats for preclinical development of drugs acting on the serotonin transporter. Int J Neuropsychopharmacol 2009;12:1021-1032.*
46. Maeda J, Morino Y, Katayama J, Horie S, Inaba K, Nishiyama A, *et al. p.2.d.002 Wf-516, a novel antidepressant with dual serotonergic activity: II. Pharmacological profiles ex vivo and in vivo to monoamine transporters in comparison with standard antidepressant. Eur Neuropsychopharmacol 2006;16:333-334.*
47. Takano A, Suhara T, Ichimiya T, Yasuno F, Suzuki K. *Time course of in vivo 5-HTT transporter occupancy by fluvoxamine. J Clin Psychopharmacol 2006;26:188-191.*
48. Tai YC, Ruangma A, Rowland D, Siegel S, Newport DF, Chow PL, *et al. Performance evaluation of the microPET focus: a third-generation microPET scanner dedicated to animal imaging. J Nucl Med 2005;46:455-463.*
49. Hildebrandt IJ, Su H, Weber WA. *Anesthesia and other considerations for in vivo imaging of small animals. ILAR J 2008;49:17-26.*
50. Tokugawa J, Ravasi L, Nakayama T, Lang L, Schmidt KC, Seidel J, *et al. Distribution of the 5-HT(1A) receptor antagonist [(18)F]FPWAY in blood and brain of the rat with and without isoflurane anesthesia. Eur J Nucl Med Mol Imaging 2007;34:259-266.*