Pharmacokinetic and Pharmacodynamic Analysis of CNS Drugs

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The elucidation of relationship between pharmacokinetics and pharmacodynamics of drugs is pivotal not only for the discovery of novel drugs but also for their optimum clinical uses. Most of clinically used drugs exert their pharmacological effects by targeting to neurotransmitter receptors and transporters in the central nervous system (CNS) and peripheral tissues. Therefore, the aim of our research is to conduct an integrated analysis of pharmacokinetics and pharmacodynamics of drugs by characterizing drug-target molecule binding under physiological and pathological conditions.

In previous studies, the binding affinities of drugs to neurotransmitter receptors and transporters have been evaluated mainly by *in vitro* experiments using crude cell membranes of native tissues and human recombinant receptor expressing cell membranes, but such *in vitro* drug-target molecule binding characteristics may not necessarily reflect *in vivo* pharmacological specificity because they do not take various pharmacokinetic and pharmacodynamic factors into account. Thus, *in vivo* characterization of drug-target molecule binding would provide more practical information for the understanding of pharmacological effects of drugs. In fact, our current studies have shown that there are significant differences among tissues (or brain regions) as well as among drugs, in the degree, duration and tissue selectivity of receptor occupancy following systemic administration of receptor targeted drugs such as 1,4-dihydropyridine (DHP) calcium channel antagonists,[1-5] $_1$-adrenoceptor antagonists,[6,7] thyrotropin-releasing hormone (TRH) analogues,[8-10] nociceptin receptor antagonists,[11] angiotensin II receptor antagonists[12] and muscarinic receptor antagonists.[13] Such *in vivo* receptor binding characteristics of these drugs were closely related to their pharmacological specificities.

In this symposium, I focus on *in vivo* receptor (or transporter) binding and CNS effects of 1,4-DHP calcium channel antagonists,[1-4] TRH analogues[8-10] and antidepressants,[14-17] in relation to their pharmacokinetics such as plasma concentration and brain uptake.

Calcium channel antagonists

Repeated oral administration of 1,4-DHP calcium channel antagonist, nimodipine in senescence-accelerated prone mouse (SAMP8) brought about an increased density of L-type calcium

channel antagonist receptors in the cerebral cortex and hippocampus accompanied by a significant improvement in the learning deficits as assessed in passive avoidance responses, but similar administration of amlodipine did not exert such effects. The subsequent *in vivo* experiments with tritiated ligands have shown that nimodipine is more extensively taken up into brain from plasma than amlodipine and binds to the receptor sites in brain parenchymal cells in a significant amount. Thus, the simultaneous measurement of pharmacokinetics and *in vivo* receptor binding in mouse brain suggests beneficial effects of nimodipine on the neurological disorders such as deficits of learning and memory.

TRH analogues

It is considered that CNS pharmacological effects by TRH and its analogues may be mediated by the stimulation of TRH receptors in the brain parenchyma. Novel TRH analogues, when injected intravenously, significantly bind to rat brain TRH receptors and that the TRH receptor occupancy is closely correlated with CNS pharmacological effects. The capillary depletion analysis in mouse brain after the intravenous injection of [^{3}H]MeTRH showed that this radioligand was distributed largely in the brain parenchyma and this distribution was significantly inhibited by co-injection of TRH and its analogues, suggesting that the transport of this agent into the brain is saturable.

Antidepressant agents

Selective serotonin reuptake inhibitors (SSRIs) such as fluvoxamine are clinically used in the treatment of depression and other psychiatric diseases. Oral administration of SSRIs (fluvoxamine, fluoxetine, paroxetine and sertraline) exerted selective binding of serotonin transporter (SERT) in mouse brain by *ex vivo* and *in vivo* experiments (evaluated by [^{3}H]paroxetine binding assay), and the binding of fluoxetine and paroxetine was of longer duration than that of fluvoxamine. The value for area under the curve (AUC) for *in vivo* SERT binding vs time in mouse brain was largest for fluoxetine among SSRIs, due to relatively longer-lasting occupation of brain SERT. The AUC value in brain SERT binding after oral administration of each SSRI was greater in the thalamus and midbrain than in the cerebral cortex, striatum and hippocampus. The peak and duration of SERT binding by these agents paralleled generally to those of marble burying behavior in mice and to their plasma concentrations. These data reveal that SSRIs bind to brain SERT *in vivo* and their pharmacological characteristics are dependent on the SERT binding activity and on the pharmacokinetics.

Continuous exposure to paroxetine at the therapeutic plasma concentration maintained by implantation of minipumps produced down regulation of [^{3}H]paroxetine binding sites in each brain region of mice. In Western blot analysis, expression levels of SERT protein in the thalamus and midbrain of these mice were significantly decreased. Thus, it has been shown that continuous

administration of paroxetine induces significant reduction of not only ligand binding sites of SERT but also the protein expression level in mouse brain. Such down-regulation of SERT may be partly related to the therapeutic effect of long-term treatment with SSRIs in human.

The pharmacological effects of extracts of Hypericum perforatum (St John's wort: SJW) were also characterized. Oral administration of SJW inhibited significantly synaptosomal [^{3}H]serotonin uptake without exerting SERT binding in mouse brain. SJW significantly inhibited marble-burying behavior and immobility time of mice in dose-dependent manner, and the time-course of attenuation by SJW of these behaviors was similar to that of inhibition of brain synaptosomal [^{3}H]serotonin uptake. These results provide the first *in vivo* evidence to suggest that the mode of antidepressant action of SJW differs from that of SSRI.

In conclusion, it is emphasized that simultaneous analysis of *in vivo* drug-target molecule binding in relation to the pharmacokinetics in the plasma and brain may be a powerful way to characterize pharmacological effects of CNS drugs.

References

1. Yamada, S. et al.: Alterations in calcium antagonist receptors and calcium content in senescent brain and attenuation by nimodipine and nicardipine. *J. Pharmacol. Exp. Ther.* 277, 721-727, 1996
2. Uchida, S. et al.: Brain pharmacokinetics and *in vivo* receptor binding of 1,4-dihydropyridine calcium channel antagonists. *Life Sci.* 61, 2083-2090, 1997
3. Yamada, S. et al.: Increase in receptor binding affinity for nimodipine in the rat brain with permanent occlusion of bilateral carotid arteries. *Life Sci.* 66, 1351-1357, 2000
4. Uchida, S. et al.: *In vivo* specific binding characteristics and pharmacokinetics of a 1,4-dihydropyridine calcium channel antagonist in the senescent mouse brain. *Pharm. Res.* 17, 844-850, 2000
5. Yamada, S.: *In vivo* receptor binding specificity of benidipine and amlodipine in mesenteric arteries of spontaneously hypertensive rats. *Life. Sci.* 70, 1999-2011, 2002
6. Yamada, S.: *In vivo* measurement by [^{3}H]tamsulosin of α_1 adrenoceptors in rat tissues in relation to the pharmacokinetics. *J. Pharmacol. Exp. Ther.* 289, 1575-1583, 1999
7. Yamada, S. et al.: In vivo demonstration of α_{1A}-adrenoceptor subtype selectivity of KMD-3213 in rat tissues. *J. Pharmacol. Exp. Ther.* 296, 160-167, 2001
8. Urayama, A. et al.: Brain receptor binding characteristics and pharmacokinetic- pharmacodynamic analysis of thyrotropin-releasing hormone analogues: *Life Sci.* 70, 647-657, 2001
9. Urayama, A. et al.: Neuroprotective effect and brain receptor binding of taltirelin, a novel

thyrotropin-releasing hormone (TRH) analogue, in transient-forebrain ischemia of C57BL/6J mice. *Life Sci.* 72, 601-607, 2002

10. Urayama, A. et al.: Blood-brain permeability of [^{3}H]-(3-methyl-His^{2})thyrotropin- releasing hormone (MeTRH) in mice: Effects of TRH and its analogues. *Drug Metabol. Pharmacokin.* 18, 310-318, 2003
11. Yamada, S. et al.: *In vitro* and *ex vivo* effects of a selective nociceptin/orphanin FQ (N/OFQ) peptide receptor antagonist, CompB, on specific binding of [^{3}H]N/OFQ and [^{35}S]GTP S in rat brain and spinal cord. *Br. J. Pharmacol.* 139, 1462-1468, 2003
12. Nozawa, Y. et al.: Receptor occupation in myocardium, adrenal cortex, and brain by TH-142177, a novel AT_1 receptor antagonist in rats, in relation to its plasma concentration and hypotensive effect. *Pharm. Res.* 15, 911-917, 1998
13. Oki, T. et al.: Muscarinic receptor binding, plasma concentration and inhibition of salivation after oral administration of a novel antimuscarinic agent, solifenacin succinate in mice. *Br. J. Pharmacol.* 145: 219-227, 2005
14. Hirano, K. et al.: In vivo identification and characterization of binding sites for selective serotonin reuptake inhibitors in mouse brain. *Life Sci.* 75, 2933-2945, 2004
15. Hirano, K. et al.: Effects of oral administration of extracts of *Hypericum perforatum* (St. John's wort) on brain serotonin transporter, serotonin uptake and behaviors in mice. *J. Pharm. Pharmacol.* 56: 1589-4598, 2004
16. Hirano, K. et al.: Relationship between brain serotonin transporter binding, plasma concentration and behavioral effect of selective serotonin reuptake inhibitors. *Br. J. Pharmacol.* 144: 695-702, 2005
17. Hirano, K. et al.: Effects of continuous administration of paroxetine on ligand binding site and expression of serotonin transporter protein in mouse brain. *Brain Res.* 1053: 154-161, 2005