PET imaging of neurogenic activity in the adult brain: Toward in vivo imaging of human neurogenesis

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ABSTRACT
Neural stem cells are present in 2 neurogenic regions, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG), and continue to generate new neurons throughout life. Adult hippocampal neurogenesis is linked to a variety of psychiatric disorders such as depression and anxiety, and to the therapeutic effects of antidepressants, as well as learning and memory. In vivo imaging for hippocampal neurogenic activity may be used to diagnose psychiatric disorders and evaluate the therapeutic efficacy of antidepressants. However, these imaging techniques remain to be established until now. Recently, we established a quantitative positron emission tomography (PET) imaging technique for neurogenic activity in the adult brain with 3-deoxy-3-[18F]fluoro-L-thymidine ([18F]FLT) and probenecid, a drug transporter inhibitor in blood-brain barrier. Moreover, we showed that this PET imaging technique can monitor alterations in neurogenic activity in the hippocampus of adult rats with depression and following treatment with an antidepressant. This PET imaging method may assist in diagnosing depression and in monitoring the therapeutic efficacy of antidepressants. In this commentary, we discuss the possibility of in vivo PET imaging for neurogenic activity in adult non-human primates and humans.

Neurogenesis occurs in 2 restricted neurogenic regions, the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ), which is adjacent to the lateral ventricles, in the adult brain throughout life.1,2 Adult neurogenesis takes place in both rodent and primate brains under physiologic conditions.3-7 Hippocampal neurogenesis has been linked to learning and memory8,9 as well as mood disorders.10-13 In addition, selective serotonin-reuptake inhibitors (SSRIs) may produce antidepressant effects through enhanced hippocampal neurogenesis.14,15 In vivo imaging of hippocampal neurogenesis may assist in diagnosing depression and in monitoring the therapeutic efficacy of antidepressants. Until now, several studies have established in vivo imaging procedures for neurogenesis in adult rodents. For instance, magnetic resonance imaging (MRI) can be used to visualize migration of neural stem or progenitor cells from the SVZ by using in vivo.16-20 However, neural stem or progenitor cells need to be labeled with MRI contrast agents via direct injection into neurogenic regions. In addition, these agents are non-selectively incorporated into both proliferating and non-proliferating cells.18 Thus, this strategy does not allow for non-invasive and quantitative evaluation of alterations in proliferative activity in the neurogenic regions of the adult brain. In contrast, positron emission tomography (PET) imaging has been used for the non-invasive detection of proliferative activity in the adult brain using 3-deoxy-3-[18F]fluoro-L-thymidine ([18F]FLT), a PET tracer.21 However, quantitative alterations in neurogenic activity have been difficult to measure, owing to lower differences in PET signals between the neurogenic regions and other regions of the adult brain. Recently, we succeeded in establishing quantitative PET imaging methods for evaluating neurogenic activity in the adult brain with probenecid, a drug transporter inhibitor at the blood-brain barrier (BBB).22 Probenecid is an inhibitor of ATP-binding cassette (ABC) transporter subfamily C members (ABCCs) and solute carrier proteins, such as organic anion transporter...
The use of probenecid resulted in enhanced brain uptake of $\text{[18F]FLT}$ in adult rats and improved $\text{[18F]FLT-PET}$ imaging for neurogenic activity.\textsuperscript{22} Until now, there have been no published studies on BBB permeability of $\text{[18F]FLT}$ with inhibitors of drug efflux transporters. To our knowledge, this is the first report demonstrating the effect of a drug transporter inhibitor at the BBB on brain uptake of $\text{[18F]FLT}$. Based on our findings, PET brain imaging may be improved by inhibition of several drug efflux transporters at the BBB.

The current study has encouraged us to establish PET imaging for proliferative activity in the hippocampus of adult primates, including monkey and human, with $\text{[18F]FLT}$ and probenecid.\textsuperscript{22} There are some differences in adult neurogenesis between rodents and primates. Indeed, the number of newborn neurons in primates appears to be several times lower than that in rodents.\textsuperscript{5,12,26-28} However, it has been reported that the relative rate of proliferative activity in the DG of hippocampus is similar between rodents and primates, including cynomolgus and rhesus monkeys when compared at the same age.\textsuperscript{29} Since $\text{[18F]FLT}$ can detect cell proliferation in neurogenic regions of adult rats under physiologic conditions,\textsuperscript{21,22} our $\text{[18F]FLT-PET}$ imaging technique with probenecid may be used to establish in vivo imaging for proliferative activity in the DG of adult monkeys. Indeed, we are attempting to establish PET imaging methods for neurogenic activity in adult rhesus monkeys. As mentioned above, there is a difference in the proportion of newly-generated neurons between rodents and primates due to maturation time in each species.\textsuperscript{28} More than 70% of newborn cells in the DG of adult rodents were NeuN+ mature neurons at 3 weeks after bromodeoxyuridine (BrdU)-labeling.\textsuperscript{14} However, only 10% and 34% of BrdU-labeled cells in macaque monkey were also immunopositive for NeuN at 6 weeks and 6 months after BrdU administration, respectively.\textsuperscript{27} Presumably, maturation time of newborn neurons in humans is longer than that in adult monkeys. It is necessary to consider these differences when establishing in vivo imaging for neurogenesis in adult primates.

Although PET and MRI are suitable for the detection of adult neurogenesis in living humans, these imaging modalities are not without limitations.\textsuperscript{30} As stated previously, MR imaging of neurogenesis in the adult brain is invasive, and currently available contrast agents are non-specifically to proliferating and non-proliferating cells. Recently, the problem of cellular specificity has been addressed using anti-CD15 antibody conjugated superparamagnetic iron oxide nanoparticles in vivo MR imaging, although this imaging technique requires direct injection of the nanoparticles into the lateral ventricles of adult rodents.\textsuperscript{31} Future studies should focus on developing non-invasive techniques for MR imaging of neural stem cells in vivo. Although, MR imaging using targeting nanoparticles allows for the assessment of neural stem/progenitor cell dynamics from the SVZ to the rostral migratory stream in vivo, PET imaging with $\text{[18F]FLT}$ allows for the specific, non-invasive monitoring of proliferating cells in neurogenic regions.\textsuperscript{21,22} Presently, however, no PET imaging methods are available for the assessment of neuronal lineage cells generated from neural stem cells because of the lack of specific PET ligands for each cell type. Therefore, to more fully evaluate the dynamics of neurogenesis in adult primates via PET imaging, future studies should focus on the development of novel PET tracers for neuronal lineage cells, and on monitoring the proliferation and differentiation abilities of neural stem cells using each PET ligand.

We have reported that the enhanced $\text{[18F]FLT}$-PET imaging technique with probenecid may be used to diagnose depression and monitor the therapeutic efficacy of antidepressants. It has been reported that the number of proliferating cells is lower in the DG of adult macaque monkeys that exhibit stress-induced depressive-like behaviors than in control animals, and that these reductions in the number of Ki67 immunopositive cells were attenuated following treatment with the antidepressant fluoxetine.\textsuperscript{32} Furthermore, these results were supported by data showing the number of doublecortin (DCX)-expressing neuronal progenitor cells in the anterior DG of both the untreated stress group and the fluoxetine-treated stressed group.\textsuperscript{32} These findings suggest that quantification of the number of newly generated neurons, as well as of the proliferating cells, may assist clinicians in diagnosing depression and in monitoring the efficacy of antidepressants. Furthermore, current research suggests that our enhanced $\text{[18F]FLT-PET}$ imaging method can be used to detect alterations in proliferative activity in adult monkeys with stress-induced depression. Analysis of DCX-positive cells in the DG of human patients between 0 and 100 y of age has suggested that the dynamics of hippocampal neurogenesis...
in humans with aging was similar to that in rodents. Postmortem analysis of the human brain has revealed that the number of proliferating cells in the SGZ of the DG is decreased in patients with psychiatric disorders, and that treatment with antidepressants stimulates the proliferation of neural progenitor cells in the DG of patients with major depressive disorder. Thus the establishment of non-invasive PET imaging methods for the evaluation of proliferative activity in the living human brain may aid clinicians in diagnosing major depressive disorder and, monitoring the therapeutic efficacy of antidepressants.

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