The Proteomics of Positron Emission Tomography

Over the past decade, research into the biology of neurodegeneration has evolved from emphasizing dysfunction of neurotransmitter systems to include investigations of protein abnormalities. This is especially clear in the study of Alzheimer's disease (AD) in which the well-known findings concerning cholinergic dysfunction that led to the first specific therapies have been augmented by research suggesting key roles for amyloid and tau in the cause and pathogenesis of the disease. Indeed, the aggregation, altered processing, and abnormal folding of proteins that may disrupt neural function is now a widespread theme that echoes throughout the study of many neurodegenerative diseases.

The application of positron emission tomography (PET) to the study of AD parallels this shift in emphasis. Although most clinicians and scientists are familiar with the use of PET to measure fundamental physiological processes such as blood flow and glucose metabolism, the strength of the technique lies in its ability to quantitatively map the distribution of a multitude of different radiolabeled tracers. Initial use of PET in the study of AD emphasized changes in glucose metabolism seen with the tracer [^{18}F]-fluorodeoxyglucose (FDG) and found metabolic deficits that are relatively specific in their predominance in temporoparietal and posterior cingulate cortex. How this pattern of abnormal metabolism should be used in the diagnosis of dementia remains somewhat controversial, but there is no doubt that the technique has yielded important data relevant to clinical diagnosis, presymptomatic detection, and prediction of decline.[1,2] Glucose metabolism is, however, relatively nonspecific in reflecting a metabolic response to a variety of different pathological processes, so the value of FDG-PET lies predominantly in the regional topography of metabolic lesions as opposed to the underlying neurochemistry itself. PET studies of AD became more neurochemically specific with the development and application of tracers for the cholinergic system. A particularly effective approach is the use of radiotracers such as [^{11}C]PMP and [^{11}C]MP4A that are hydrolyzed by the enzyme acetylcholinesterase and remain trapped in tissue, reflecting cholinergic innervation and function.[3,4] This method has shown reduction in brain cholinesterase levels of approximately 30% in AD patients that is related to disease progression[5] and that can be used to asses the extent of cholinesterase inhibition by existing pharmacological therapy.[6]

In this issue of the *Annals*, Klunk and colleagues report the results of the next step in the evolution of PET in the application to AD: development of a radioligand targeted to the amyloid protein itself.[7] The compound, *N*-methyl-[^{11}C]-2-(4′-methylaminophenyl)-6-hydroxybenzothiasole (nicknamed PIB), is structurally related to the thioflavin-T molecule, a dye that has long been used to label amyloid in histological studies. Klunk and colleagues present a substantial amount of data that support the use of this compound as a marker of brain amyloid deposition. Previous work by this group demonstrated rapid blood–brain barrier permeability, with labeling of both amyloid angiopathy and plaques in transgenic mouse models of AD,[8] as well as in vitro binding to AD brain and synthetic amyloid fibrils but not to neurofibrillary tangles (NFTs).[9] The work reported here extends these observations to in vivo human studies.

Klunk and colleagues demonstrate several points that substantiate using this PET radiotracer for imaging humans with AD. First, they have shown displaceable, specific binding to postmortem AD brain but not to control brain. Plasma metabolism produces only metabolites that will not cross the blood–brain barrier, so the radioactive signal observed in the brain is caused only by the compound and not a less specific signal from a metabolite. The dynamic PET data show that tracer washout is slower in AD patients than controls and that these differences are most pronounced in brain regions known to contain substantial amyloid deposits. In cerebellum and white matter, where little fibrillar amyloid is present, the tracer behaves similarly in patients and controls. The pattern of tracer uptake, shown by using a standardized uptake value in which tissue concentration is normalized to the injected amount of radioactivity and the patient's body mass, is also considerably different between patients and controls. This pattern largely reflects our current knowledge about the topography of amyloid deposition and does not seem consistent with binding to NFTs. The differences between patients and controls seen using PIB were similar to, but greater than, those seen using FDG.

The data available on this compound are impressive, reflecting a decade of research and development beginning with molecular drug design and including extensive in vitro and animal testing. Not surprisingly, there is still more that we need to know. An important series

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of questions could not be answered by this study because technical factors limited the authors' ability to mathematically model the binding of the compound. This modeling is important in evaluating a new PET tracer and helps to differentiate specific tracer binding to a target from nonspecific effects such as reduced tracer delivery secondary to reduced blood flow. Fortunately, if the reduced blood flow that is characteristic of AD were a major factor one would expect reduced uptake in AD patients, whereas uptake was increased in AD patients compared with controls. Nevertheless, changes in blood flow could affect the relative accumulation of PIB in different brain regions, accounting for the pattern of distribution seen in this study. More precise quantitation of tracer binding will be needed to address many fundamental biological questions.

The question of whether PET amyloid imaging with this tracer can diagnose AD also remains unanswered by this study. The authors selected an especially mild group of AD patients, most of whom had scores on the Mini-Mental State Examination of higher than 25. With a mean age of slightly older than 65 years, these patients were also younger than the typical AD patient. The use of relatively young controls matched in age to the patients, and the finding that young and older controls did not differ in tracer retention, would seem to avoid the problem of contamination of the control group with early AD. However, the selection of such mildly affected AD patients, some of whom showed tracer uptake more typical of controls, unfortunately leaves unanswered the question of whether discrepancies between PIB binding and clinical diagnoses reflect inaccurate diagnosis or an insensitive PET technique. Of course, the study was not designed as a test of the diagnostic accuracy of PIB imaging.

Finally, Klunk and colleagues also note some uncertainty as to the exact species of amyloid to which the tracer binds. Although it does appear to bind to fibrillar forms that include both neuritic and nonneuritic plaques, it is not certain whether the compound binds to soluble and oligomeric forms of amyloid. Recent evidence suggests that these forms of amyloid may be important in producing clinical symptoms and early functional deficits.[10,11]

The potential applications of this tracer are considerable. Careful clinical studies will be needed to guide clinical applications, whereas answers to questions about quantitation and binding will be important for basic research. Validation for diagnostic use will require at minimum larger studies with more typical patients and some degree of neuropathological confirmation. The use in presymptomatic diagnosis and prediction is another important potential application that may require more accurate quantitative models and a better understanding of the precise molecular targets of the probe. There is also considerable interest in using imaging technologies as surrogate outcome measures in clinical trials. When disease-modifying therapies that interfere with amyloid deposition enter clinical trials, this radioligand could provide important information about efficacy to shorten the duration of such trials and lessen the sample size. Finally, the ability to accurately quantify the deposition of brain amyloid will provide a tool for answering many fundamental questions about differences between normal aging and AD, and an examination of the amyloid hypothesis of AD pathogenesis itself.

The development of radiotracers for the detection of abnormal protein deposition in the brain is an intense area of investigation in many laboratories. Another compound, [^{18}F]1,1-dicyano-2-[6-(dimethylamino)-2-naphthalenyl]propene or FDDNP has been studied in several in vitro preparations as well as in humans and appears to label amyloid, NFTs, and prion plaques.[12–14] New compounds are under study, including those with potential as single-photon emission computed tomography imaging agents, and nonradioactive compounds for use with magnetic resonance imaging have been investigated. Although the developmental work is substantial, it is likely that additional compounds for AD and we hope new compounds with specificity for abnormal proteins in other neurodegenerative disorders such as α-synuclein in Parkinson's disease and tau in frontotemporal dementia will arise. The apparently straightforward question of which imaging agent is "best" will depend on the specific question being asked and is likely to be unsettled as long as new compounds are being developed and detailed data about their characteristics accumulate. What is clear is that the ability to measure the deposition of proteins that may play a fundamental role in the pathogenesis of neurodegeneration has expanded the role of PET imaging as a molecular neuroscience technique that will have important consequences for basic research and clinical care.

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