Advances in neuroimaging over the past 2 decades are products of breakthroughs in imaging technology, developments of more powerful computers and image-processing software, and expanding knowledge in basic and clinical neuroscience. In addition to the insights into normal brain structure and function that such methods provide and the information that can be gained from disease-related changes in structure and function, the promise of achieving diagnostic specificity through neuroimaging lies with the potential identification of pathognomonic proteins. Recent advances in imaging β-amyloid plaques, one of the hallmarks of Alzheimer disease, offer such a technological breakthrough and the possibility for more efficient assessment of antiamyloid interventions as well as specific noninvasive diagnostic capabilities.

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In the last several decades of the 20th century and the first several years of the 21st, research and the clinical application of neuroimaging have proceeded at a dizzying and accelerating rate. Structural imaging was initially limited to skull and spine radiographs and subsequently to inference about what was happening in the brain by analyzing the patterns of arterial, capillary, and venous blood flow on cerebral angiography. The advent of the computed tomography scan in the 1970s allowed visualization of brain parenchyma for the first time, and advances in hardware, computer power, and imaging programs led to improved resolution. Magnetic resonance imaging added to the bounty of new structural imaging techniques in the 1980s and also progressed through increased imaging resolution and new sequences that led to better visualization of white matter, water diffusion, and other pathological disturbances. Techniques for the rapid assessment of blood flow advanced functional imaging and the localization of brain activity or function. Magnetic resonance spectroscopy continues to develop as another method for the noninvasive assessment of brain metabolism in development, aging, and disease.

The development of positron emission tomography (PET) has followed a similar rapid evolution since implementation in the 1970s. These advances include improved detector systems, increases in computer processing power and programs to use such power, and advances in radiochemistry. These advances in turn led to breakthroughs in our understanding of cerebral blood flow, energy metabolism, and neurotransmitter function in humans both with and without neurological disease. Patterns of abnormalities in blood flow or glucose metabolism, although nonspecific, have provided significant diagnostic aid in Alzheimer disease, dementia with Lewy bodies, and frontotemporal dementia. The ability to devise imaging ligands that bind to central nervous system enzymes, receptors, transporters, or other components of neurotransmitter systems has been...
the special province of PET. Alterations in these PET markers over the course of time has aided diagnosis and added information about the progression of disease.

Recently, PET research has begun to focus on the ability to image the abnormal proteins associated with specific neurodegenerative diseases. The ability to image the amyloid plaques and neurofibrillary tangles of Alzheimer disease (AD), the Lewy bodies of Parkinson disease or dementia with Lewy bodies, or other pathognomonic proteins would enable unprecedented accuracy of diagnosis; it would also allow staging of disease and a basis for the efficient evaluation of therapeutic interventions to halt the accumulation of, decrease the levels of, or remove abnormal proteins.

For amyloid, the potential benefits of a pathology-driven imaging approach are the following:

- Improvements in early diagnosis and the possibility of a presymptomatic diagnostic biomarker, depending on the time course of amyloid deposition
- Improved understanding of the natural history of amyloid deposition and insights into the pathophysiology of AD, along with a potential tool to further evaluate amyloid cascade hypothesis
- The capability to directly measure the effects of newly developed anti amyloid therapies (eg, secretase inhibitors, immunotherapy, and “plaque breakers”)

The ability to directly measure a pathological change believed to be intimately related to the pathophysiology of the disease might have advantages over less specific structural or metabolic measures in all of these areas, but the advantage is clearest in the ability to directly measure the efficacy of antiamyloid therapies. Toward this end, our group set out to develop PET tracers specifically for the β-amyloid peptide (Aβ) in plaques and cerebrovascular amyloid in AD and related conditions. Our goal was to apply PET technology to detect and quantify a specific pathological change in the brain.

**Aβ-SPECIFIC RADIOTRACERS**

The design and biological evaluation of agents for PET and single-photon emission computed tomography to image Aβ plaques in the living brain have been reviewed recently. The most successful radiotracers to date for these purposes have proven to be relatively small molecules (<600 d), which display better in vivo pharmacokinetic imaging properties than large proteins such as Aβ peptides and monoclonal antibodies. Well-known Aβ-specific histological dyes, such as Congo red and thioflavin T, have provided a starting point for the development of these agents, from which many series of analogues have been synthesized and evaluated as Aβ imaging agents for PET and single-photon emission computed tomography. However, the ionic charges on Congo red and thioflavin T prevented good penetration of the blood-brain barrier by the radiotracer analogues. Neutral, lipophilic derivatives were necessary to achieve sufficient quantities of the radiotracer in the brain for subsequent PET or single-photon emission computed tomography imaging studies. Although removal of the ionic charge from Congo red analogues lowered the binding affinity of the derivatives for Aβ, deletion of the charge from thioflavin T dramatically increased the binding affinity of the derivatives for Aβ. Further derivatization of these neutral analogues of thioflavin T (termed benzothiazole anilines) indicated that substitution of the 6-methyl group with a 6-hydroxyl group provided a potent Aβ ligand with excellent in vivo pharmacokinetic imaging properties. This 6-hydroxyl–substituted benzothiazole aniline is known as Pittsburgh Compound B (PIB) (Figure 1). When radiolabeled with carbon 11, PIB provides a radioligand for PET imaging studies of Aβ in the brains of living human subjects. Pittsburgh Compound B retains the highly fluorescent properties of its parent, thioflavin T, and has been used to image Aβ in the brains of living transgenic mice using multiphoton microscopy techniques.

Critical to the demonstration of the binding targets of benzothiazoles were binding assays that used homogenates of postmortem brain because these assays could be performed using the low nanomolar concentrations of amyloid probe that are attainable during in vivo PET studies. In brain areas known to contain high concentrations of Aβ deposits, tissue homogenate studies showed greater than 10-fold more binding of benzothiazole compounds to AD brain compared with age-matched control brain. In contrast, no increase in binding was seen in areas of AD brain without fibrillar amyloid deposits (eg, cerebellum). Although neurofibrillary tangles could be stained with fluorescent benzothiazoles applied at a high concentration (1 µM), a 1-nM concentration of benzothiazole compounds resulted in no increase in binding over background in brain tissue homogenates that contained large numbers of tangles but no plaques (ie, Braak II control brain transentorhinal cortex). This suggested that under in vivo PET conditions (at nM tracer levels), benzothiazoles cannot detect tangle deposits and that the signal observed is primarily due to Aβ deposits. Finally, multiphoton microscopy studies of PIB binding in transgenic mouse models of amyloid deposition clearly demonstrated specific retention of PIB in plaque and cerebrovascular amyloid deposits, confirming both brain entry and plaque specificity in a living animal model of AD.

**IN VIVO PHARMACOKINETIC MODELING**

A primary objective of PIB PET modeling studies has been to define a simple and valid method for the in vivo measurement of amyloid deposition across the AD spectrum, one that can be readily applied in the clinical setting. Because postmortem binding assays have shown PIB binding characteristics to be consistent with classical li-
gand-binding interactions,3 our analytic approaches were those well-established for the quantification of PET imaging studies of ligand-receptor binding. Data were acquired in AD and control subjects in a fully quantitative manner that allowed flexibility for subsequent analysis by multiple approaches that vary in complexity, accuracy, and reliability. These approaches yield PIB retention measures that range from fully quantitative (distribution volume) to semiquantitative (standardized uptake values) and include the following: compartmental modeling (fully dynamic PET, arterial input: distribution volume), graphical methods (simplified dynamic, with and without arterial input: distribution volume), and late-scan PET uptake measures (standardized uptake values). Good agreement has been found across the analytic methods with respect to the regional pattern of PIB retention in AD, distinct differences in PIB retention between AD and control subjects in areas known to contain amyloid in AD, and similar levels of nonspecific PIB retention in AD and control subjects.7 The latter similarity in reference PIB retention supports our investigation of image-based analyses that eliminate the necessity for arterial blood sampling. In fact, good agreement has been found between the fully quantitative arterial-based methods and simplified reference-based analyses that allow rapid generation of image maps of PIB retention. The next step is to apply the regional results to validate volumetric statistical assessments of parametric images maps of PIB retention that are based on the anatomical standardization of each individual’s magnetic resonance and PIB PET data to a common reference. Preliminary parametric statistical assessments have provided results that agree with the regional evaluations. These stepwise analyses of the PIB PET method should lead to the identification of a simple and valid method that will be feasible for routine use and provide a pragmatic compromise between methodological accuracy and precision.

HUMAN AMYLOID IMAGING STUDIES

Friedland et al8 reported the first attempt to image amyloid using a radiolabeled anti-Aβ antibody Fab fragment. Insufficient brain entry of the large macromolecule made this approach impractical. The second in vivo human amyloid imaging study used the tracer 2-(1-[6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl] ethyldene) malononitrile (FDDNP) to quantify amyloid in 9 AD patients and 7 controls.8 The time-activity data from an AD patient included in that study indicated that, at late time points (90-120 minutes), the absolute retention of the tracer FDDNP in neocortical areas exceeded that in the reference region, the pons, by 10% to 15%. The area of highest retention at late time points was the hippocampus/amygdala/entorhinal cortex region, an area that exceeded the reference region by approximately 30%. The tracer FDDNP was reported to image both plaques and neurofibrillary tangles; however, validation for the latter claim is based on tissue-staining studies. Relevance of such staining studies for activity in vivo in humans must be interpreted with caution because the histological procedures employ greater than 1000-fold higher concentrations of the amyloid probe typically used in tracer PET studies.

The third attempt to study human in vivo amyloid imaging used PIB and was presented in preliminary form by Engler et al10 followed by the full report in 2004.4 This first human PIB study included 16 patients with mild AD (Mini-Mental State Examination scores, 18-28) and 9 healthy control subjects. Three of the 9 controls were young (21 years old) to guarantee the absence of plaques in these subjects. There was a robust difference in PIB retention between the AD patients and the healthy controls. Compared with controls, AD patients showed marked retention of PIB in areas of the brain association cortex known to contain large amounts of amyloid deposits in AD. In AD, PIB retention increased most prominently in the frontal cortex (>2-fold; P<.002). Equally important, retention was equivalent in AD patients and controls in areas known to be relatively unaffected by amyloid deposition (such as the subcortical white matter, pons, and cerebellum; P>.2). Studies in young and old healthy controls showed very low PIB retention in cortical areas, independent of subject age. Pittsburgh Compound B retention correlated inversely with cerebral glucose metabolism in the parietal cortex, determined with 2-[F-18]fluoro-2-deoxy-D-glucose (FDG) across all subjects. The group difference in PIB retention was of greater magnitude than FDG detected hypometabolism in all brain areas (Figure 2).

As we have previously suggested,4 it is important to avoid the circular reasoning inherent in the association of amyloid deposition with both the diagnosis and the etiology of AD. Therefore, at the outset, it might be best not to equate amyloid deposition with clinical diagnosis (independent of the subject’s clinical status). Instead, it might be best to first think of PIB retention more fundamentally as a method to detect and quantify brain “β-amyloidosis,” a phrase first used in reference to AD by Glenner.11 Several fundamental and unbiased questions can then be asked regarding the following:

- The cross-sectional variation in β-amyloidosis across the spectrum of dementia severity in AD
- The natural history of β-amyloidosis, its onset relative to clinical symptoms of dementia, and its progression in individual patients across the course of the dementia
- The correlation of β-amyloidosis with clinical diagnosis
- The usefulness of β-amyloidosis as a surrogate marker of efficacy for antiamyloid therapeutics

Perhaps the most important short-term role of amyloid imaging will be in the development of several new classes of antiamyloid therapies, which recently have entered or soon will enter human trials (eg, passive immunization, γ-secretase and β-secretase inhibitors, and β-sheet breakers). All of these therapies focus on decreasing the amyloid load in brain. Without a surrogate biomarker to assess the efficacy of these therapeutic agents on their intended central nervous system amyloid target, one cannot properly interpret the outcome of a therapeutic trial. For example, a negative clinical outcome in a therapeutic trial of passive immunization (expected to
remove β-amyloid from brain) would be interpreted very differently if no effect on amyloid load was observed at a given dose than if a widespread reduction in amyloid load was coupled with a negative clinical outcome. Likewise, a positive clinical outcome could not be accepted as proof of the “amyloid cascade hypothesis” until a significant effect on amyloid deposition could be coupled with the clinical effect.

Amyloid imaging technology holds promise as a diagnostictic technique as well. The added value of diagnostic potential will not be fully realized until an effective treatment exists for AD. At that time, the ability to identify very early (and perhaps presymptomatic disease) will become essential for early and optimal initiation of therapy. Additionally, assessment of amyloid load changes over time will likely be a necessary surrogate marker of treatment efficacy.

To prepare for these upcoming applications of amyloid imaging, we are initiating fundamental studies, including the following:

- Optimization of the pharmacokinetic modeling analysis of the in vivo data and simplification of the imaging protocol
- Cross-sectional studies of amyloid deposition across all stages of disease severity in AD and mild cognitive impairment
- Studies assessing amyloid deposition in normal aging and relating the asymptomatic presence of amyloid to the likelihood of developing AD at a later time
- Longitudinal studies of amyloid load progression in AD and mild cognitive impairment
- Assessing the contribution of amyloid deposition to dementia in Parkinson disease and dementia with Lewy bodies

Studies also are being initiated in special populations such as nondemented Down syndrome subjects and asymptomatic members of families with autosomal dominant AD gene mutations. In addition, preliminary studies are being initiated in early-phase therapeutic trials of putative antiamyloid drugs. We also are actively working to develop a fluorine-18-labeled derivative of PIB. This derivative will allow for wider distribution and application of this amyloid imaging technology.

In summary, the ability of PET to identify and quantitate β-amyloid in vivo in humans has significant implications for presymptomatic detection, differential diagnosis, and therapeutic interventions in AD. Certainty of diagnosis is not only important clinically; it also increases the certainty of diagnosis when exploring other behavioral or biological studies in the disease. Visualization of an abnormal protein in a neurodegenerative disease in vivo provides both proof of principle and powerful motivation to develop neuroimaging agents for other neurodegenerative diseases.

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