Neuroimaging of Vessel Amyloid in Alzheimer's Disease[a,b]

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ABSTRACT: Despite extensive recent advances in understanding Alzheimer's disease (AD) we are unable to noninvasively establish a definite diagnosis during life and cannot monitor the cerebral deposition of amyloid β protein (Aβ) in living patients. We evaluated the use of 10H3, a monoclonal antibody Fab targeting Aβ protein 1-28 labeled with Tc-99m. Six subjects with probable AD were studied using single-photon emission computed tomography (SPECT) at times from 0–24 hours following injection. Curves of radioactivity in blood demonstrate a half-life of the injected Fab of 2–3 hours. Images show uptake around the head in the scalp or bone marrow in all subjects. There is no evidence of cerebral uptake of the antibody. Scalp biopsies in all six patients demonstrate diffuse staining with 10H3 of the scalp, a pattern indistinguishable from that found in controls. Evidence of amyloid deposition in the scalp in AD is not seen with other anti-Aβ antibodies, suggesting that 10H3 is cross-reacting with another protein. Further studies with anti-Aβ antibodies will require longer-lived radionuclides to detect cerebral uptake at later times after injection to allow for complete clearance from the blood. Alternately, imaging using labeled Aβ itself may provide a means for noninvasive targeting of cerebral amyloid.

Remarkable advances have been made in our understanding of the mechanisms of amyloid β (Aβ) protein synthesis, cleavage, and aggregation, as well as pathways for tau protein phosphorylation responsible for neurofibrillary tangle formation. Three important genes with mutations causing Alzheimer's disease (AD) have now been discovered (chromosomes 21, 14 and 1). There is also an important risk factor gene, believed to encode apolipoprotein E, on chromosome 19 which may be responsible for up to 30–40% of the attributable risk of AD. However, clinicians

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still have no method available for the definite diagnosis of AD other than brain biopsy. Also, there is no method for evaluation of the progression of Aβ protein deposition in living patients. Patients with AD have intense accumulation of Aβ protein in senile plaque cores and in vessel walls in arteries, arterioles, and capillaries.[1,2] Amyloid angiopathy was observed in 83% of 117 brains from AD patients in a study from the Consortium to Establish a Registry for AD.[3] Vessel amyloid is accompanied by changes in vessel morphology, including thickening of the basement membrane and destruction of the smooth muscle layer.

Brain imaging has been widely used in studies of AD. All current methods address aspects of the disease that are not central to the key pathogenetic mechanisms. We can study regional glucose metabolism, cerebral blood flow, oxygen metabolism, neuroreceptor density, and neurotransmitter uptake in the disease, but these important indices of brain function do not reflect primary disease mechanisms in AD. A method to allow for quantitative detection of cerebral amyloid deposits is needed. Anti-amyloid antibodies showing great affinity for the Aβ protein have added insights to our understanding of amyloid deposition in the brain in studies performed *in vitro*. We have hypothesized[4–12] that intravenously injected anti-amyloid antibodies will have access to vascular sites of amyloid deposition because of the extensive presence of Aβ in vessel walls.[13] We do not believe that the blood–brain barrier in AD is sufficiently broken down to allow for intravenously administered antibody to enter the brain parenchyma and label amyloid in neuritic plaque cores. Our hypothesis is that injected anti-amyloid antibodies will label sites of amyloid beta deposition in the endothelium of brain vessels on the blood side of the blood–brain barrier.[11,12]

One considerable issue in this approach is to develop a ligand which has rapid blood clearance so that the binding of the antibody to vessel walls can be detected after clearance of the label from the intracerebral blood pool (which amounts to approximately 5% of brain intracranial volume). For this reason we have fragmented the anti-amyloid antibody to its Fab fragment in order to diminish its half-life in the blood.

METHODS

Antibody Development

Antibody 10H3 was developed in a murine ascites system targeting amyloid Aβ 1-28 using a synthetic antigen.[11,14,15] The antibody was evaluated, prepared, and mass-produced to GMP standards as previously described.[14,15] Antibody 10H3 was chosen because of excellent characteristics in labeling plaque and vessel amyloid *in vitro*.[11,14,15]

Fractionation and Technetium Labeling

Fab fragments were obtained using enzymatic methods and were labeled with generator-produced Tc-99m using the diamine dimercaptide bifunctional chelating system, as described by Fritzberg and associates[16] and implemented by the NeoRx Corp., Seattle, WA.

Toxicity, Biodistribution, and Flow Cytometry

Hematology, blood chemistry and histopathology was tested in adult Sprague-Dawley rats. Binding of the Fab to human white and red cells was tested using flow cytometry.

Subjects

Six patients were studied [mean age 73 (SD 10) years]. Three were male and three female. All patients had probable AD according to NINCDS-ADRDA criteria. Mini-mental Status Examination testing showed a mean score of 15 (SD 8, range 4–25). Autopsy material taken from control subjects was also studied histochemically.

Imaging and Biodistribution

Single-photon emission computed tomography (SPECT) was done using a Trionix scanner (Ohio). Labeled Fab was injected intravenously, mean dose 4.1 mg protein (SD 1.0, range 2.5–5), 33 mCi (SD 6.3, range 22–38.5). Biodistribution was determined by sampling blood at rapid periods after injection for 1 hour and then at ½-hour intervals for 2 hours, and then at less frequent intervals for 24 hours. Patients were hospitalized in the Clinical Research Center at University Hospitals of Cleveland.

Scalp Biopsies

Scalp biopsy specimens were obtained in all six subjects using a standard dermatological punch procedure following local anesthesia. Tissues were fixed in paraformaldehyde lysin periodate, paraffin-embedded, and cut into 6-μM sections. Immunostaining was performed with anti-amyloid antibodies 10H3 (Aβ 1-28), 4G8 (Aβ 17-23), 3160 (Aβ 1-40), 6E10 (Aβ 1-17), and R1280 (Aβ 1-40). Sections were also counterstained with hematoxylin and eosin. Fixed but sucrose-protected cryostat sections were also immunostained in parallel to overcome paraffin-embedding artifacts.

IND #BB-5059 was received from the U.S. Food and Drug Administration, Division of Biological, for this investigation. Informed consent was obtained from all subjects or families, and the study was approved by the Investigational Review Board of the University Hospitals Cleveland.

RESULTS

The half-life of the injected technetium labeled 10H3 Fab is 2–3 hours in the plasma. SPECT images obtained immediately after injection demonstrate presence of the label in the cerebral blood pool in all subjects with diffuse uptake in the cerebrum. Images obtained at later intervals up to 17–24 hours demonstrate accumulation of the label in the scalp or bone marrow around the surface of the head. Because of the spatial resolution characteristics of SPECT, we are unable to localize

the precise anatomical site of this activity. There is no clear uptake of the label in the brain parenchyma even at longer periods after injection (24–36 hours).

Scalp biopsy specimens demonstrate diffuse nonspecific staining with 10H3, but Aβ is not detected using any of the other anti-Aβ antibodies. Scalp tissue from controls also shows diffuse staining with 10H3 but no labeling with the other antibodies. Hair follicles are stained with 4G8 in both AD cases and controls. Hair follicle staining with 4G8 appears to represent binding to pigment.

DISCUSSION

A cardinal feature of AD is the deposition in cortex of large quantities of fibrillar Aβ protein.[1-3] The presence of this protein in a β-pleated sheet comformation is used post mortem to establish the diagnosis, along with the detection of other pathological features, including neuropil threads, neurofibrillary tangles, granulovacuolar degeneration, and neuronal cell loss. There is no method available to image any of these cerebral pathologic features noninvasively during life. A novel approach is the use of immunochemistry in living patients by means of monoclonal antibodies targeting disease proteins, such as Aβ.

In vivo radioimmunodetection has been successful in imaging sites of metastatic deposits in colon and breast cancer and melanoma.[17-19] A major problem with the use of antibody imaging in neurology is the blood–brain barrier, which generally excludes large molecules such as antibodies from entry. Workers dating back to Scholz (1938), who first noted amyloid infiltration in vessel walls in AD ("drusige Entartung"), have concluded from anatomical studies that blood–brain barrier function in AD should be abnormal.[2] Degeneration of endothelium and thickening of the basement membrane with vascular degeneration has been observed in small vessels in AD, suggesting the presence of barrier changes.[1] However, studies of blood–brain barrier functioning in living patients has not provided evidence of abnormalities.[20,21]

It has been proposed that the presence of normal cerebrospinal fluid total protein in AD is evidence for a normal blood–brain barrier. However, the relationship between cerebrospinal fluid and serum protein levels is dependent upon cerebrospinal fluid flow, and cerebrospinal fluid flow has been found to diminish with aging.[22] The integrity of the blood–brain barrier in Alzheimer's disease remains an open issue.

Anatomical studies have shown the proximity of Aβ deposits to the endothelium of capillaries, suggesting that circulating anti-amyloid antibodies would have access to antigen sites without the necessity of passing through the blood–brain barrier. The diamine dimercaptide method was used to label the monoclonal antibody 10H3 with technetium-99m with high specificity and stability. An Fab fragment was used because it is less immunogenic and has a shorter circulation time than intact antibody. The 6-hour physical half-life of Tc-99m may not be sufficient to allow for detection of label in brain beyond 18–24 hours.

We found uptake of labeled anti-amyloid antibody 10H3 around the head. Scalp biopsies did not demonstrate evidence of Aβ infiltration of scalp. Conflicting results regarding the presence of Aβ in skin in AD and controls have been reported.[23]

Use of antibodies labeled with longer-lived radionuclides such as iodine-131 may allow for detection of intravascular binding of monoclonal antibodies to cerebral amyloid in Alzheimer's disease. A further approach is suggested by novel experiments demonstrating binding of labeled Aβ itself to cerebral amyloid in

AD.[24-29] Zlokovic and colleagues have reported that Aβ coupled to apolipoprotein J crosses the blood–brain barrier better than native Aβ or Aβ coupled to apolipoprotein E.[28] Ghilardi *et al.* infused aged squirrel monkeys with I-125-labeled Aβ via the carotid artery and demonstrated labeling of cerebral amyloid deposits.[29] Labeling of cerebral amyloid *in vivo* has also been demonstrated in squirrel monkeys with an intrathecally administered anti-amyloid antibody.[30]

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