Mechanism of Amyloid Removal in Patients With Alzheimer Disease Treated With Gantenerumab

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Background: Gantenerumab is a fully human anti-β-monoclonal antibody in clinical development for the treatment of Alzheimer disease (AD).

Objectives: To investigate whether treatment with gantenerumab leads to a measurable reduction in the level of Aβ amyloid in the brain and to elucidate the mechanism of amyloid reduction.

Design: A multicenter, randomized, double-blind, placebo-controlled, ascending-dose positron emission tomographic study. Additionally, ex vivo studies of human brain slices from an independent sample of patients who had AD were performed.

Setting: Three university medical centers.

Patients: Patients with mild-to-moderate AD.

Intervention: Two consecutive cohorts of patients received 2 to 7 infusions of intravenous gantenerumab (60 or 200 mg) or placebo every 4 weeks. Brain slices from patients who had AD were coincubated with gantenerumab at increasing concentrations and with human microglial cells.


Results: Sixteen patients with end-of-treatment positron emission tomographic scans were included in the analysis. The mean (95% CI) percent change from baseline difference relative to placebo (n=4) in cortical brain amyloid level was −15.6% (95% CI, −42.7 to 11.6) for the 60-mg group (n=6) and −35.7% (95% CI, −63.5 to −7.9) for the 200-mg group (n=6). Two patients in the 200-mg group showed transient and focal areas of inflammation or vasogenic edema on magnetic resonance imaging scans at sites with the highest level of amyloid reduction. Gantenerumab induced phagocytosis of human amyloid in a dose-dependent manner ex vivo.

Conclusion: Gantenerumab treatment resulted in a dose-dependent reduction in brain amyloid level, possibly through an effector cell–mediated mechanism of action.


Videos available online at www.archneurol.com
of brain Aβ amyloid as measured by [11C]PiB PET. Additionally, we report local effects of gantenerumab on brain magnetic resonance imaging (MRI) and provide an integrated analysis of results from the 2 imaging modalities. Furthermore, we link imaging results to data from an ex vivo assay in brain slices, all in an effort to elucidate the mechanism by which gantenerumab reduces the level of brain amyloid.

**METHODS**

**PATIENTS**

Data reported here are from a PET substudy of a multiple ascending dose (MAD) trial with gantenerumab. The clinicaltrials.gov identifier for the MAD study is NCT00531804. Complete methods and results from the MAD study will be reported separately; only select data related to the PET data are included here. To be eligible for the PET substudy, patients had to fulfill all entry criteria of the MAD study, with the following key criteria: 50 to 90 years of age, probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria, a Mini-Mental State Examination score between 16 and 26 (inclusive), an MRI scan consistent with AD, and a modified Hachinski ischemia score of 4 or less. Stable symptomatic treatment of AD was allowed. Eligibility criteria specific to the PET substudy excluded patients who had been exposed to radiation in the past year or planned such exposure. Patients signed a written informed consent (cosigned by the patient’s next of kin or caregiver, if required by local regulations) prior to screening. The PET substudy was reviewed and approved by an independent ethics committee at each site as well as by the respective health authorities.

**GANTENERUMAB**

Gantenerumab is a human IgG1 with a high affinity for fibrillar Aβ. The original clone was derived from the MorphoSys HuCAL-Fab1 phage display Human Combinatorial Antibody Library ( Martinsried, Germany) and optimized by in vitro affinity maturation. Specificity for human Aβ present in senile plaques was demonstrated ex vivo by immunohistochemical staining of human brain sections at low picomolar concentrations. In vivo, gantenerumab crosses the blood-brain barrier and binds specifically and dose-dependently to Aβ plaques in PS2APP transgenic mice. Long-term treatment with gantenerumab over 5 months significantly decreased the amyloid plaque load in PS2APP mice assessed immunohistochemically.3,7

**RANDOMIZATION AND BLINDING**

A subset of patients from 2 cohorts (a 60-mg cohort followed by a 200-mg cohort) of the MAD study participated in the PET substudy. Patients in each cohort were randomly assigned to receive either gantenerumab or placebo with a drug-to-placebo ratio of 4:1. Study site and sponsor personnel were blinded to treatment.

**MAD STUDY**

Patients were to receive up to 7 intravenous infusions of gantenerumab or placebo every 4 weeks. DNA samples were obtained for APOE genotyping. Magnetic resonance imaging monitoring included a 3-dimensional T1-weighted, T2*-weighted, and a fluid-attenuated inversion recovery (FLAIR) sequence. The instruments used for the clinical assessments included the Alzheimer’s Disease Assessment Scale–cognitive subscale, the Mini-Mental State Examination, a modified neuropsychological test battery, disability assessment for dementia, adverse event reporting, and laboratory tests.

**PET SUBSTUDY**

Positron emission tomographic imaging was performed at 3 sites using ECAT EXACT HR+ cameras (Siemens, Erlangen, Germany). Approximately 370 MBq of [11C]PiB (prepared as per local procedures) were administered as an intravenous bolus. The PET data were collected 60 to 90 minutes after the tracer injection. Frame-to-frame realignment was used to correct for any motion before a sum image was created. Frame-to-frame realignment was used to correct for any motion before a sum image was created. [11C]PiB summed images were coregistered to the patient’s baseline MRI scan, and MRI and PET data were spatially normalized into Montreal Neu-
rological Institute space where a volume-of-interest template was used to define target regions and a cerebellar cortex reference region. The same set of volumes of interest was applied across all PET scans for each patient, and target region–to–reference region standard uptake value ratios (SUVRs) were computed. The quantitative analysis focused on a cortical composite volume of interest comprising a volume-weighted average of frontal, parietal, lateral temporal and sensorimotor, anterior, and posterior cingulate cortices. To determine whether focal findings observed on MRI scans were related to levels of amyloid clearance, the central MRI reader manually outlined findings of interest on the MRI scan (FLAIR sequence). Resulting binary masks were overlaid on the corresponding PET images, and the SUV percent change was calculated in the areas of focal MRI signal change. All image analysis was performed by individuals blinded to study treatment allocation.

STATISTICAL ANALYSIS

The sample size was pragmatic rather than based on statistical power estimations. Approximately 35 patients from 4 dose cohorts were expected to participate. However, only 2 cohorts participated; a lower dose cohort (20 mg) was not included in the power estimations. Approximately 35 patients from 4 dose cohorts were expected to participate. However, only 2 cohorts participated; a lower dose cohort (20 mg) was not included in the

The prespecified analysis plan included within- and between-group comparisons of mean SUV change from baseline to post-baseline times to be evaluated with paired and 2-sample t tests. However, owing to the small number of patients who actually contributed to the analysis, nonparametric techniques were applied. For between-group comparisons, a nonparametric analysis of covariance was used. To assess the dose-response relationship, linear regression was applied on the baseline-adjusted residuals of the percent change values. Owing to the exploratory nature of the analysis, nominal P values are presented without any adjustment for multiplicity.

The change over time in the regional SUV was assessed in terms of simple subtraction (SUVRBL–1) and percent change ([(SUVRFU–SUVRBL)/SUVRBL] × 100), which represents change at follow-up (FU) relative to total [11C]PiB signal at baseline (BL). In addition, to allow for direct comparison with data from a recent publication, percent change was calculated as follows: [(SUVRBL–SUVRe0)/SUVRBL] × 100. The constant of 1 is subtracted from the denominator because this is the background, nonspecific component. This constant gets canceled in the numerator. The resulting percent change represents change relative to a specific [11C]PiB signal at baseline (ie, SUVRe0–1).

Table 1. Clinical Key Baseline Characteristics of 18 Patients With Mild-to-Moderate Alzheimer Disease

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>60 mg of Gantenerumab</th>
<th>200 mg of Gantenerumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline scan</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Age at baseline, mean (SD), y</td>
<td>62.8 (3.5)</td>
<td>70.9 (8.1)</td>
</tr>
<tr>
<td>MMSE score at baseline, mean (SD)</td>
<td>21.0 (2.5)</td>
<td>21.8 (3.6)</td>
</tr>
<tr>
<td>APOE carriers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e3/e3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>e3/e4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>e4/e4</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: MMSE, Mini-Mental State Examination.

One patient in the 60-mg cohort did not consent to genotyping.

Table 2. Baseline and Change From Baseline to End of Treatment in the Cortical Composite Standard Uptake Value Ratio

<table>
<thead>
<tr>
<th>Patients, No.</th>
<th>Placebo b</th>
<th>60 mg of Gantenerumab c</th>
<th>200 mg of Gantenerumab d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline SUVR</td>
<td>2.18 (0.17)</td>
<td>2.86 (0.73)</td>
<td>2.86 (0.63)</td>
</tr>
<tr>
<td>Actual SUVR at end of treatment</td>
<td>2.42 (0.19)</td>
<td>2.88 (0.67)</td>
<td>2.59 (0.77)</td>
</tr>
<tr>
<td>Actual SUVR, mean of individuals’ changes from baseline at end of treatment</td>
<td>0.24 (0.15)</td>
<td>0.03 (0.24)</td>
<td>-0.27 (0.45)</td>
</tr>
<tr>
<td>% Change score, mean of individuals’ % changes from baseline at end of treatment</td>
<td>20.9 (15.6)</td>
<td>5.3 (19.7)</td>
<td>-14.9 (20.3)</td>
</tr>
</tbody>
</table>

Abbreviation: SUVr, standard uptake value ratio.

aActual and percent change (specific carbon 11–labeled Pittsburgh Compound B signal); owing to early termination of dosing in the 200-mg cohort, not all patients received all 7 infusions of gantenerumab.
bTwo patients received all 7 infusions, 1 patient received 2 infusions, and 1 patient received 5 infusions.
cAll patients received all 7 infusions.
dOne patient received 2 infusions, 2 patients received 3 infusions, 2 patients received 4 infusions, and 1 patient received 5 infusions.
eTwo patients without end-of-treatment scans are not included.

IN VITRO INTERACTION STUDY

To evaluate whether gantenerumab interferes with hydrogen 3–labeled [3H]-PiB binding to amyloid, an in vitro study was performed. Consecutive frozen sections from brains of 2 patients who had AD but were not in the clinical trial (Banner Sun Health Research Institute, University of Arizona, Sun City) were preincubated with up to 5000 ng/ml of gantenerumab to saturate antibody binding sites on amyloid plaques and were subsequently incubated with 1nM of [3H]-PiB to determine total bind-
ing. Consecutive sections were incubated with [3H]-PiB in the presence of an excess unlabelled ligand (1µM) to determine non-specific binding. Images were assessed quantitatively using a Fujiﬁlm BAS-TR2025 phosphoimager (Tokyo, Japan).

**EX VIVO PHAGOCYTOSIS ASSAY**

To evaluate gantenerumab’s ability to clear amyloid plaques via phagocytosis, an ex vivo study was performed. Human primary microglia cells were freshly isolated from healthy human brain tissue obtained during tumor surgery (University Hospital Zurich, Switzerland). After gentle homogenization, microglia cells were enriched, removed from ﬂasks, analyzed by a ﬂuorescence-activated cell sorter using anti-CD45, and used in the phagocytosis assay if more than 95% of the cells were positive for CD45.

Cortical brain tissue from patients who had AD but were not in the clinical trial (Braak stage VI, at Banner Sun Health Research Institute) was cryosectioned at a nominal thickness of 20 µm and placed onto culture dishes (Biocoat 40629; BD Biocoat, San Jose, California). Consecutive sections were preincubated with and without different concentrations of gantenerumab before the microglia cells were seeded at 1.5 x 10^6 cells/mL and cultured at 37°C for 3 days. After fixation, Aβ plaques were detected by staining with an N-terminal speciﬁc mouse monoclonal antibody BAP-2 conjugated to Alexa Fluor 488 dye (Molecular Probes, Eugene, Oregon). An unrelated human IgG1 (PHP010; AbD Serotec, Raleigh, North Carolina) antibody served as a control.

Time-lapse live-cell imaging was done over 12 hours at an image frequency of every 10 minutes. Gantenerumab conjugated to Alexa Fluor 488 dye (Molecular Probes, Eugene, Oregon) was preincubated at 5 µg/mL, and microglia cells seeded, at conditions described above in a culture chamber attached to a Leica SP2 confocal microscope (Leica Microsystems, Buffalo Grove, Illinois).

**RESULTS**

**PET STUDY**

Reduction in Brain Amyloid Level After Treatment With Gantenerumab

In this PET study, 18 patients were randomly assigned to receive either placebo or gantenerumab (60 or 200 mg intravenously) (Figure 1 and Table 1). Owing to the early termination of dosing in the 200-mg cohort, not all patients received all 7 infusions (Table 2). Although the mean MMSE score was similar across groups at baseline, patients in the placebo group were younger and had lower brain Aβ amyloid levels (Tables 1 and 2). Hence, a statistical evaluation of the data was adjusted for baseline SUVR.

Table 2 and Figure 2 summarize the cortical composite SUVR at the end of treatment and the means of individuals’ changes from baseline. The actual mean (SD) changes were 0.24 (0.15) for the placebo group, 0.03 (0.24) for the 60-mg group, and −0.27 (0.45) for the 200-mg group. The mean (SD) percent change from baseline over total PiB signal was 11.0% (7.6%) for the placebo group, 2.1% (10.3%) for the 60-mg group, and −9.4% (14.0%) for the 200-mg group. The mean (SD) percent change from baseline over the specific PiB signal was 20.9% (15.6%) for the placebo group, 5.3% (19.7%) for
Figure 3. Effect of gantenerumab on amyloid load as indexed by standard uptake value ratios (SUVRs) using carbon 11–labeled Pittsburgh Compound B (\([11C]\)PiB) positron emission tomography. Scatterplot shows percent change from baseline (specific \([11C]\)PiB signal) in cortical composite SUVR over gantenerumab doses for all patients with an end-of-treatment scan who received gantenerumab (60 or 200 mg) or placebo every 4 weeks. The dose-response relationship is indicated by the linear regression line (% change in amyloid = 12.81 – 0.13 \(dose\)) of the baseline-adjusted percent change residual value (vertical axis) vs actual dose of gantenerumab (horizontal axis).

Figure 4. Effect of gantenerumab on amyloid load as indexed by standard uptake value ratios (SUVRs) using carbon 11–labeled Pittsburgh Compound B (\([11C]\)PiB) positron emission tomography. The median SUVR percent changes from baseline (specific \([11C]\)PiB signal) by brain region are shown for patients who received infusions of intravenous gantenerumab (60 or 200 mg) or placebo every 4 weeks.

Figure 5. Magnetic resonance imaging (MRI) scans from an \(APOE\) \(\varepsilon4\) homozygous patient. Images shown represent scans at baseline (A), during treatment (B), and after treatment (C) that were acquired using a fluid-attenuated inversion recovery sequence. The new area of hyperintensity on the scan performed during treatment (B) is most prominent in the right temporal lobe (arrow) and is consistent with inflammation or vasogenic edema. It first appeared on the scheduled MRI scan 2 weeks after the second drug infusion, was progressive for 6 weeks, and subsequently spontaneously completely resolved by week 17 (C).
the 60-mg group, and −14.9% (20.3%) for the 200-mg group. The observed mean (95% CI) treatment differences from the placebo group in percent change over the specific PiB signal were −15.6% (95% CI, −42.7% to 11.6%) for the 60-mg group and −35.7% (95% CI, −63.5% to −7.9%) for the 200-mg group. Adjusting for baseline SUVR, we found that a nonparametric analysis of covariance on this percent change suggested that the 200-mg group differed from the placebo group (P = .06). The dose dependency of the amyloid-reducing effect was indicated by the nonparametric linear regression analysis on the baseline-adjusted percent change values over the specific PiB signal: slope of −0.13 (r² = 0.29; P = .03) (Figure 3).

Figure 6. Integrated analysis of amyloid positron emission tomography (PET) and magnetic resonance imaging (MRI). The PET and MRI scans from the 2 patients (ie, patients A and B) are shown. The baseline standard uptake value ratio (SUVR) images are superimposed on the baseline MRI scans (A and C), and the binary masks of the MRI (fluid-attenuated inversion recovery) findings as outlined by the expert reader are superimposed on the baseline structural MRI scans (B and D). The end-of-treatment SUVR maps are superimposed on the baseline MRI scans (E and G), and the difference maps of SUVRs at the end of treatment minus baseline are superimposed on baseline MRI scans (F and H). The late follow-up SUVR maps are superimposed on the baseline MRI scans (I and K), and the difference maps of SUVRs at late follow-up minus baseline are superimposed on the baseline MRI scans (J and L). Crosshairs indicate positioning of the MRI finding.
Changes were consistent across regions (Figure 4), except in the pons, which is a brain area known to have very limited amyloid deposition.4 Changes in subcortical white matter may indicate some gray matter contamination of this volume of interest. Although dose-dependent reductions in the level of amyloid were observed, no consistent treatment effects on cognitive measures were noted in this small group of patients treated for a short period of time. Moreover, individual changes in cognitive measures did not correlate with changes in levels of amyloid.

Greatest Reduction in Level of Amyloid in Areas of MRI-Detected Abnormality

Focal MRI signal changes were observed in 2 APOE ε4 homozygous patients following 2 and 4 doses of 200 mg of gantenerumab, respectively. Findings were most conspicuous on the FLAIR sequence and consistent with inflammation or vasogenic edema (Figure 5). They resolved spontaneously after discontinuation of dosing. Both patients also developed microhemorrhages (images not shown), and one of them was mildly symptomatic (headache, dizziness, gait instability, and tremor). All other patients (these include those in the MAD study) with such MRI changes were asymptomatic. Areas of high signal on FLAIR were often colocalized with prominent decreases in the SUVR (Figure 6). Patient A showed no overall reduction in the SUVR following 4 doses of gantenerumab (200 mg); however, a localized area of decreased SUVR in the area of the FLAIR signal in the right temporal lobe is shown in Figure 6E and F. This localized area of amyloid reduction was still present in the posttreatment PET scan acquired 6 months after complete resolution of the MRI finding (Figure 6I and J). Patient B (who happened to have the largest decrease in SUVR) showed an overall reduction in SUVR following 2 doses of gantenerumab (200 mg) with a unilateral amyloid reduction in the left caudate nucleus (Figure 6G and H), an area of focal high-FLAIR signal. This effect appeared essentially unchanged in the posttreatment PET scan performed 8 months after the MRI finding had completely resolved (Figure 6K and L). In both patients, amyloid reduction was greater in areas of FLAIR signal compared with the prespecified cortical composite volume of interest (Figure 7).

IN VITRO INTERACTION ASSAY

In vitro studies demonstrated that there was a lack of interference between [3H]-PiB and gantenerumab, and therefore these studies support the notion that in vivo changes in the SUVR that are based on [11C]PiB binding truly reflect changes in fibrillar plaque amyloid load.

EX VIVO PHAGOCYTOSIS ASSAY

A decrease in Aβ amyloid plaque in sections of brain that were incubated with microglia cells was dependent on the concentration of gantenerumab, with a slight effect at 50 ng/mL of gantenerumab and substantial plaque clearance at 500 and 5000 ng/mL (Figure 8). Live-cell imaging showed that a removal of fluorescent-labeled gantenerumab bound to amyloid deposits occurred within hours through active intracellular uptake by migrating microglia adjacent to amyloid plaques (Figure 8; videos, http://www.archneurol.com).

COMMENT

There is a large body of evidence showing that [11C]PiB PET accurately indexes the load and location of Aβ amyloid in the brain.10-12 Our study demonstrates that 2 to 7 months of treatment with gantenerumab led to dose-dependent amyloid reduction in the brains of patients with AD. Additionally, our findings in the placebo-treated patients support previous reports indicating that amyloid load continues to increase in many patients with mild-to-moderate AD.3,13 This is in contrast with earlier data suggesting that amyloid load had reached a steady state in these patients.14 Estimates of percent change may vary between studies depending on how they are derived. Rinne et al.,2 using the same method focused on here that relates change to specific rather than total [11C]PiB binding, reported a similar increase in the level of amyloid in placebo-treated patients with AD. Moreover, they reported that 18 months of treatment with bapineuzumab resulted in a decrease in amyloid level but no dose response.3 Given the small sample sizes in the study by Rinne et al and in the study presented here, a comparison of the magnitude of the effect is not robust, although it may be noted that the effect of gantenerumab appeared rapidly, after 2 to 7 monthly infusions.

Treatment with bapineuzumab can result in reversible vasogenic edema,1 more recently described as “amyloid-related imaging abnormalities,”15 observed more frequently in carriers of the APOE ε4 genotype.4 We observed
similar MRI findings in 2 patients (both carriers of APOE ε4/ε4) treated with 200 mg of gantenerumab. Although MRI cannot determine with certainty the underlying pathophysiology, this focal high-FLAIR signal was frequently colocalized with areas of higher amyloid reduction. Such local effects on amyloid PET could not be attributed to poor tracer penetration due to acute, local edema because they were still apparent 6 to 8 months after complete resolution of the MRI finding.

Several mechanisms for brain amyloid reduction by antiamyloid antibodies have been suggested. They include effector cell–mediated phagocytosis and direct dissolution of amyloid.16 Our observation of more prominent amyloid reduction in areas of increased FLAIR signal may provide clues as to the mechanism by which gantenerumab clears amyloid: (1) Microglial cells contain very low levels of Aβ in untreated patients with AD,17 whereas postmortem studies following treatment with AN1792 suggest that antiamyloid antibodies lead to an increase in Aβ phagocytosis.17,18 Furthermore, results from the ex vivo assay reported herein support the hypothesis that gantenerumab clears amyloid plaques via Fc re-
ceptosomes, degradation as demonstrated for differentiated human macrophages. The colocalization of the focal FLAIR signal and amyloid reduction may be due to an exaggerated microglial response resulting in locally perturbed vascular permeability. (2) Direct dissolution of aggregated Aβ and subsequent Aβ drainage along the perivascular pathways may result in a transient increase in cerebral amyloid angiopathy. Accordingly, patients who received active Aβ immunization treatment were reported to have a significant increase in the level of Aβ42 and Aβ40 to a lesser extent) in cerebral vessel walls at autopsy. When plaques are dissolved rapidly, clearance mechanisms may get saturated with a possible result of vasogenic edema. Also, in this instance, one might expect the MRI finding to more likely occur in or adjacent to areas with greater amyloid clearance, and both mechanisms may result in microhemorrhages.

In summary, although both clearance mechanisms may occur in parallel, the ex vivo data reported herein implicate phagocytosis as a more likely mechanism of amyloid reduction by treatment with gantenerumab. The FLAIR hyperintensities may be seen as instances of excessive pharmacological activity due to a high dose or more susceptible individuals (eg, carriers of the APOE ε4 genotype). Indeed, a lesser degree of Aβ amyloid reduction relative to placebo was observed in other brain areas and with a lower dose of gantenerumab in the absence of detectable FLAIR hyperintensities. This suggests that gantenerumab-induced amyloid lowering can be achieved without significantly perturbing vascular permeability through inflammation or blockage of Aβ clearance pathways when appropriate dosing is selected.

The main limitations of the present study are its small size and the unequal distribution of amyloid load at baseline between the treatment and placebo groups. Although statistical analysis methods were chosen to address these limitations, any conclusions are provisional in nature. Additionally, it is still unclear whether any reduction in brain amyloid level will translate into clinical efficacy. A phase 2 clinical trial is under way to investigate whether a clinical benefit can be achieved in gantenerumab-treated patients with prodromal AD.

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Online-Only Material: The eTable and videos are available at http://www.archneurol.com.

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REFERENCES