Reduction of Amyloid in the Brain and Retina After Treatment With IVIG for Mild Cognitive Impairment

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Abstract
Objective: To assess whether intravenous immunoglobulin (IVIG) in subjects with mild cognitive impairment (MCI) results in a reduction in amyloid in the central nervous system (CNS).
Methods: Five subjects with MCI underwent baseline Florbetapir positron emission tomography and retinal autofluorescent imaging. All were administered IVIG (Octagam 10%) at 0.4 g/kg every 14 days for a total of 5 infusions. After 3 months, standard uptake value ratio (SUVR) and amyloid retinal deposits were reassessed.
Results: Three subjects had a reduction in amyloid SUVR and all 5 subjects had a reduction in amyloid retinal deposits in at least 1 eye.
Conclusions: A short course of IVIG over 2 months removes a measurable amount of amyloid from the CNS in persons with MCI.

Keywords
intravenous immunoglobulin, mild cognitive impairment, Alzheimer’s disease, amyloid, Aβ, PET

Introduction
Alzheimer disease (AD) is a neurodegenerative disorder that causes cognitive decline and brain atrophy associated with the pathologic accumulation of cerebral amyloid. Cerebral amyloid can be quantified using F-18-Florbetapir positron emission tomography (PET) which has been shown in postmortem investigations to have a highly significant correlation with β-amyloid plaque deposition, making this a reliable quantitative marker of amyloid load in the human brain.¹

Semi-quantitative Florbetapir PET has been shown to have up to an 88% sensitivity and 81% specificity for AD.² Semi-quantitative assessments of Florbetapir uptake demonstrated global standard uptake value ratio (SUVR) cutoff values in probable AD of 1.39 ± 0.24 and 1.17 ± 0.27 in mild cognitive impairment (MCI) compared to control groups, where cutoff values were 1.05 ± 0.16.³ The SUVR has been shown to be elevated in the anterior and posterior cingulate as well as precuneus in AD when compared to normal controls and greater in the precuneus, median frontal, and posterior cingulate in AD when compared to MCI.⁴,⁵

Another part of the central nervous system (CNS), the retina, has also been shown in postmortem histologic studies to contain amyloid β plaques in concentrations that correspond to brain amyloid burden across the AD spectrum, with retinal plaque structures ranging from 10 μm to 50 μm in diameter.⁶,⁷ These amyloid plaque structures can be identified using retinal autofluorescent (AF) imaging techniques.⁶,⁸

Intravenous immunoglobulin (IVIG) contains naturally occurring polyclonal antibodies to amyloid and has been investigated as a potential disease-modifying strategy for AD.⁹-¹² Animal models have demonstrated that IVIG crosses the blood–brain barrier reduces β-amyloid from the brain when analyzed with ex vivo and in vivo assays and induces modest improvements in memory.¹³-¹⁷

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A phase III trial of mild-to-moderate AD included an exploratory substudy which showed a reduction in amyloid SUVR of 0.062 using Florbetapir PET after 18 months of IVIG treatment 0.4 g/kg every 2 weeks. Unfortunately, this phase III study failed to show beneficial effects on cognition or function. Additional feasibility concerns about the cost and supply of IVIG have contributed to the recent pause in clinical investigations of IVIG for AD.

A retrospective study suggested a possible preventative strategy with the observation that prior treatment with IVIG in nondemented individuals was associated with a reduced risk of developing AD dementia. We investigated this type of strategy of using low doses of IVIG in patients with MCI. Patients were randomized to receive either 0.4 g/kg of IVIG or 0.9% saline solution every 2 weeks for 2 months (5 infusions totaling 2g/kg). The results demonstrated a significant reduction in brain atrophy and reduced conversions to AD dementia in the IVIG group compared to placebo at 1 year; these effects waned by year 2. The purpose of this study was to determine whether a short treatment of IVIG in patients with MCI results in a quantifiable amyloid biomarker reduction in the CNS using brain amyloid imaging and retinal amyloid imaging.

### Methods

This was a single-center, open-label, proof-of-mechanism study conducted at a nonacademic medical center memory clinic. Patients were 3 men and 2 women 50 to 85 years of age diagnosed with MCI due to AD based on National Institute on Aging and Alzheimer’s Association (NIA-AA) criteria and supported by global clinical dementia rating (CDR) ≤0.5 (Table 1). Patients underwent baseline Florbetapir PET and had retinal AF levels measured using an image-processing algorithm based on published and unpublished data. All patients received infusions of IVIG (Octagam 10%) 0.4 g/kg every 14 days for a total of 5 infusions. Repeat PET and retinal AF measures were performed 3 months after the first infusion.

Florbetapir PET images were analyzed using the syngo.MI Neurology on the syngo via platform (Siemens Healthineers, Knoxville, Tennessee). With cerebellar uptake as the reference, SUVR was calculated at both time points in each patient for anterior cingulate, frontal, parietal, posterior cingulate, precuneus, and temporal lobes based on automatically generated regional volumes of interest. In addition to regional ratios, global cerebral-to-cerebellar SUVR was calculated.

The AF images of the superior retina were obtained using the commercially available Retia AF ophthalmic camera. All pre- and post-IVIG images were obtained using the same device. The Retia (Centervue, Padova, Italy) illuminates using a blue light-emitting diode at a 450 nm wavelength and collects light above 500 nm wavelength. This combination identifies AF due to amyloid β deposits. The retinal AF images were processed using software Food and Drug Administration (FDA) cleared for measurement of AF in the retina. The software input is a set of AF images of the superior retina. It completes a series of image-processing steps to assess image quality, align and combine images to improve signal-to-noise characteristics, identify a standard region of interest, and segment AF pixels. The FDA-cleared software reports total pixels and identifies total spots a coefficient of variation of 11.6%. A spot count output measure was to quantify the retinal amyloid load. Study data were collected and managed using the Research Electronic Data Capture (REDCap) electronic data capture tools hosted at Sutter Medical Center, Sacramento.

### Table 1. Demographic and Cognitive Characteristics of Participants.

| Patient | Age | Sex | Education, Years | BMI | Vascular Comorbidities | CDR-Global | CDR-SB | MMSE |
|---------|-----|-----|------------------|-----|------------------------|------------|--------|------|
| 1       | 79.25 | Male | 12-16           | 21.92 | 0                      | 0.5        | 1.5    | 29   |
| 2       | 76.71 | Male | >16             | 23.46 | 3                      | 0.5        | 3      | 24   |
| 3       | 75.91 | Male | 12-16           | 27.09 | 2,3,4                  | 0.5        | 2      | 27   |
| 4       | 74.14 | Female | 12-16        | 26.51 | 0                      | 0.5        | 2      | 25   |
| 5       | 65.47 | Female | >16           | 18.77 | 0                      | 0.5        | 2      | 27   |

Abbreviations: BMI, body mass index; CDR, clinical dementia rating; MMSE, mini-mental state examination; SB, sum of boxes.
Results

The mean interval between the baseline imaging (Florbetapir PET and retinal AF) and the first infusion of IVIG was 8 (standard deviation [SD] = 8) days, and the interval between the last infusion and the posttreatment imaging (Florbetapir PET and retinal AF) was 33 (SD = 6) days.

Brain amyloid imaging with Florbetapir PET showed that the global SUVR for patients 1, 2, and 4 was notably less at 3 months than at baseline (Figures 1 and 2, and Table 2). The mean SUVR for patient 5 was the same as baseline and was slightly increased in patient 3. In the 2 patients (3 and 5) in which the mean SUVR was not reduced, there was some evidence of reduction of amyloid in specific brain regions: temporal and frontal regions in patient 3 and temporal, parietal, and posterior cingulate regions in patient 5. Among the 6 brain

| Table 2. Amyloid PET Standard Uptake Value Ratio for Patients at Baseline and Posttreatment Following 5 Infusions of 0.4 g/kg Intravenous Immunoglobulin. |
|---------------------------------|----------------|----------------|----------------|--------------------|----------------|
| Patient ID 12345 | Anterior Cingulate | 1 | 2 | 3 | 4 | 5 |
| Anterior Cingulate | Baseline | 1.51 | 1.63 | 1.4 | 1.76 | 1.7 |
| Anterior Cingulate | Posttherapy | 1.45 | 1.54 | 1.42 | 1.73 | 1.75 |
| Frontal lobe | Baseline | 1.52 | 1.44 | 1.19 | 1.61 | 1.33 |
| Frontal lobe | Posttherapy | 1.48 | 1.47 | 1.18 | 1.57 | 1.42 |
| Parietal lobe | Baseline | 1.27 | 1.27 | 1.03 | 1.48 | 1.3 |
| Parietal lobe | Posttherapy | 1.23 | 1.25 | 1.08 | 1.36 | 1.29 |
| Posterior cingulate | Baseline | 1.74 | 1.74 | 1.49 | 1.89 | 2.08 |
| Posterior cingulate | Posttherapy | 1.73 | 1.65 | 1.49 | 1.82 | 2.02 |
| Precuneus | Baseline | 1.72 | 1.66 | 1.44 | 1.97 | 1.91 |
| Precuneus | Posttherapy | 1.68 | 1.69 | 1.47 | 1.87 | 1.92 |
| Temporal lobe | Baseline | 1.65 | 1.5 | 1.31 | 1.72 | 1.7 |
| Temporal lobe | Posttherapy | 1.6 | 1.48 | 1.3 | 1.64 | 1.62 |
| Global | Baseline | 1.57 | 1.54 | 1.31 | 1.74 | 1.67 |
| Global | Posttherapy | 1.53 | 1.52 | 1.32 | 1.66 | 1.67 |

Abbreviation: PET, positron emission tomography.

Figure 2. Amyloid positron emission tomography (PET) brain imaging in patients 1 to 5 at baseline and 3 months after treatment with intravenous immunoglobulin (IVIG).

Figure 3. Change in retinal amyloid autofluorescent (AF) 3 months after treatment with intravenous immunoglobulin (IVIG).
regions assessed, the posterior cingulate SUVR was highest at baseline in all patients. The most significant decreases in SUVR after IVIG were in the posterior cingulate and temporal regions (Table 2).

Retinal AF imaging data show a reduction in small spot counts in at least 1 eye of all 5 patients 3 months after IVIG treatment compared to baseline (Figures 3 and 4, and Table 3). Right eye images were excluded in patient 3 due to interference of the eye lid. There was a spot count increase in the right eye of patient 4; however, this eye was noted to have ophthalmologic disease that impeded the accuracy of spot counts. The left eye of patient 5 could not be measured due to a cataract.

Discussion

There are no FDA-approved disease-modifying treatments to target the underlying pathophysiology of AD. This small proof-of-mechanism study is the first to demonstrate evidence of amyloid clearance in patients with MCI 3 months after an initial IVIG infusion and 1 month after the fifth and final infusion. Three of the 5 IVIG-treated patients demonstrated evidence of amyloid clearance from the brain, and all 5 patients demonstrated evidence of amyloid clearance from the retina in at least 1 eye. This biomarker evidence of amyloid reduction is noteworthy, especially given the short duration of treatment with IVIG.

This study supports the existing evidence that amyloid clearance is part of the mechanism of action of IVIG for AD. The IVIG also modulates inflammation and has been shown to contain tau-antibodies. These multiple mechanisms of action may prove to be the key to a successful treatment approach to AD and should be further investigated.

Reductions of brain amyloid have also been reported in monoclonal antibody studies after 1 year of treatment in patients with MCI or mild AD. However, phase III monoclonal antibody studies have failed to demonstrate evidence of improved cognitive function. This may be due to the overly specific mechanism of action of monoclonal antibodies. Intravenous immunoglobulin contains polyclonal variants of naturally occurring antibodies against amyloid, which may increase its effectiveness for AD. Post-IVIG cognitive testing was not included in this proof-of-mechanism study; however, we previously published results of a larger IVIG study in MCI which demonstrated 1-year preservation of cognitive function and delay in conversion to AD dementia in patients treated with a short course of IVIG compared to placebo. It was also observed in our prior publication that the beneficial effects of IVIG appeared to wane after 1 year.

Intravenous immunoglobulin has consistently been shown to be safe in previous clinical trials. There has been no evidence of vasogenic cerebral edema or microhemorrhages which have been observed in monoclonal antibody treatment trials. An animal model confirmed that IVIG treatment was not associated with T-cell inflammation or microhemorrhages. Considering the converging evidence, IVIG is a safer immunotherapeutic strategy than monoclonal antibodies.

Table 3. Retinal Amyloid Autofluorescence Spot Count at Baseline and Posttreatment Following 5 Infusions of 0.4 g/kg Intravenous Immunoglobulin.

| Patient ID | 1   | 2   | 3   | 4   | 5   |
|-----------|-----|-----|-----|-----|-----|
| Right eye |     |     |     |     |     |
| Baseline  | 612 | 158 | 110 | 138 |
| Posttherapy | 504 | 122 | 168 | 102 |
| Left eye  |     |     |     |     |     |
| Baseline  | 557 | 174 | 141 | 125 |
| Posttherapy | 433 | 130 | 103 | 108 |

*Not valid due to interference of eye lid.

Unable to measure due to cataract.

Figure 4. Retinal amyloid autofluorescent (AF) in patients 1 to 5 at baseline and 3 months after treatment with intravenous immunoglobulin (IVIG).
This study provides evidence that low-dose IVIG over a short period of time removes a measurable amount of amyloid from the CNS in patients with MCI, which is consistent with the desired treatment effect for AD. This proof-of-mechanism study and previous investigations suggest that IVIG remains a viable treatment strategy if given in the predementia stage of AD. A larger trial with annual booster infusions of IVIG for MCI is warranted.

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