MR Spectroscopy for Assessment of Memantine Treatment in Mild to Moderate Alzheimer Dementia

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Abstract. Objectives: Magnetic Resonance Spectroscopy (MRS) may provide a precise and reliable assessment of the extent and severity of neural tissue loss caused by various diseases. In particular, the N-Acetyl Aspartate (NAA) and Creatine (Cr) ratio has been found to be an indicator of the degree of neuronal loss in Alzheimer’s disease (AD). Memantine is thought to benefit the AD brain by stabilizing the NMDA receptors on neurons in turn reducing excitotoxicity. Despite its effectiveness in treating moderate to severe AD, memantine has not had similar success in the treatment of mildly demented AD patients. The objective of this study was to test whether memantine would slow or prevent the loss of neurons in mild to moderate AD patients. Methods: A double-blind placebo-controlled study was designed to measure the effect of a year-long course of memantine in patients with a probable AD diagnosis with mild to moderate dementia. The primary outcome measure was stipulated to be change in MRS NAA/Cr ratio in inferior parietal cortex in memantine relative to the placebo treatment condition. The secondary outcome measures were changes in cognitive and function scale scores. Results: This pilot study failed to demonstrate a benefit of memantine on the primary outcome measure, the inferior parietal NAA/Cr ratio, or the secondary outcome measures. Conclusions: More studies are needed to determine the effect of memantine on regions of the brain significantly affected by AD pathology.

Keywords: Alzheimer disease, dementia, magnetic resonance spectroscopy, memantine, cognition, N-acetylaspartate, creatine

INTRODUCTION

Cognitive and functional measures are accepted surrogate markers of Alzheimer’s disease (AD) severity. However, neuroimaging techniques, such as Magnetic Resonance Spectroscopy (MRS), can provide a more direct, precise, and reliable assessment of AD-related damage to brain tissue [1]. MRS measures neurochemicals, such as N-Acetyl Aspartate (NAA) and Creatine (Cr), and the NAA/Cr ratio reflects neural tissue volume. These neurochemical measurements are indicators of neuronal loss in AD, and clinical diagnosis of AD and frontotemporal lobar degeneration (FTLD) [2–4]. These measurements are additionally used to assess the efficacy of AD medication trials [5, 6] and correlate with the severity of neurofibrillary pathology and fibrillary tau density after death [5, 7].

While cholinesterase inhibitors are the most well established medications in AD treatment, memantine...
has been effective in the treatment of moderate to severe AD [8]. Memantine works by stabilizing the NMDA receptors in neurons of the cerebrum thus reducing excitotoxicity. Memantine’s utility in treating moderate to severe AD has not been consistent in studies of patients with mild AD using standard cognitive and functional measures to assess cognitive decline [9]. However, neuroimaging techniques are a more direct measure of AD pathology, and thus, more likely to demonstrate the effect, if any, that memantine has on the AD pathological progression.

We examined the effect of a year-long course of memantine in patients with a diagnosis of probable AD with mild dementia. Specifically, we hypothesized:

1) Memantine would result in less reduction of the NAA/Cr ratio in mild AD patients taking the drug for one year relative to patients taking placebo.
2) Slowing of the neuronal loss would correspond to a smaller decline in cognitive function in the treatment group.

METHOD

Patients

Mild to moderately demented patients with a probable AD diagnosis and their caregivers were recruited and consented. The Stanford University IRB approved the use of human subjects for this study. The clinical trials database at Stanford University has the records of this study, and this database is linked to “clinicaltrials.gov” with the “unique protocol ID” #95722. Patients had to be in stable health and able to comply with all procedures. Patients were excluded for Parkinson’s disease, any MRI contraindications, certain neurologic or psychiatric conditions (e.g., seizures, clinically significant stroke, head trauma, major psychiatric disorder) or other medical or laboratory findings or medications rendering them unsuitable for an investigational trial.

Seventeen patients met screening criteria initially, but four patients were excluded during the treatment phase due to defibrillator implant, eyeliner tattoo, severe medical illness unrelated to the study, and diagnosis change to corticobasilar degeneration. Of the 13 eligible and randomized patients scanned at the baseline and study termination, 7 on memantine, 6 on placebo completed the study, having the repeat scans an average of 54 weeks after baseline (Table 1).

Study procedures

Patients underwent a medical history review and physical and neurological exam, laboratory tests, and cognitive assessment at the screening visit. Soon after, patients had a baseline evaluation including the MRS scan, symptom review, and neuropsychiatric measures. Patients were randomized to memantine or placebo arms by the un-blinded pharmacist and study medication was dispensed.

To assess possible safety concerns, health status, and medication compliance, brief treatment visits were performed at months 1, 3, 6 and 9 and telephone checks were done six weeks after each visit.

The final visit procedures repeated those done at screening and baseline, including MRS scan, medical and laboratory evaluation, and cognitive and clinical assessment.

MRS

Images were acquired on a 3T Excite GE MRI scanner. On a sagittal scout scan, the AC-PC (anterior commissure–posterior commissure) line was determined to position a horizontal scan 1 cm above the AC-PC line. Three spectroscopic data sets were obtained from 2 x 2 x 2 cm voxels in the left cerebral cortex, inferior parietal, posterior cingulate, and occipital (Figure 1 shows the location of inferior parietal region, placed in a far lateral position just behind the insula to maximize inclusion of cortex in the voxel), using a spin-echo series of TR/TE 2000/35 msec with each preselected region of interest for point-resolved spectroscopy (PRESS). Data processing was performed using the fully automated PROBE/SV quantification tool (General Electric Medical System, Milwaukee, WI). During the second scanning session (1 year later) voxel positions were selected with reference to the structural scans from the first data acquisition.

Each of the 5 spectral areas associated with NAA, Cr, Choline (Cho), myoinositol (mI), and H2O was quantified by Marquardt-Levenworth curve fitting over that line region. Before curve fitting, line widths were normalized, and a Lorentzian-to-Gaussian transformation was performed. Cr was designated as the reference moiety. Data were analyzed as metabolite (NAA, Cho, and mI) to Cr ratio. This convention minimizes errors.
Table 1

<table>
<thead>
<tr>
<th>Demographic/Cognitive Measure</th>
<th>Memantine (n=7)</th>
<th>Placebo (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>76.5 ± 9.6</td>
<td>75.4 ± 6.3</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15.4 ± 3.4</td>
<td>13.2 ± 1.7</td>
</tr>
<tr>
<td>Gender</td>
<td>14% female</td>
<td>67% female</td>
</tr>
<tr>
<td>White</td>
<td>71%</td>
<td>67%</td>
</tr>
<tr>
<td>Asian</td>
<td>29%</td>
<td>33%</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>14%</td>
<td>0%</td>
</tr>
<tr>
<td>Dose of Memantine</td>
<td>7 (90%)</td>
<td>6 (67%)</td>
</tr>
<tr>
<td>MMSE</td>
<td>19.9 ± 4.8</td>
<td>21.8 ± 3.1</td>
</tr>
<tr>
<td>ADAS-Cog</td>
<td>41.5 ± 10.2</td>
<td>40.2 ± 8.4</td>
</tr>
<tr>
<td>ADL</td>
<td>56.9 ± 16.7</td>
<td>68.2 ± 5.1</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>Baseline</td>
<td>1.47 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Year 1</td>
<td>1.62 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>ADAS-Cog</td>
<td>44.67 ± 10.30</td>
</tr>
<tr>
<td></td>
<td>Year 1</td>
<td>45.75 ± 7.99</td>
</tr>
</tbody>
</table>

1 Memantine: During the course of the study, subjects were supplied with either memantine tablets or placebo provided by the sponsor (Forest Laboratories). After the screening visit, the pharmacist randomized subjects into one of two groups: Treatment (T; target dose 10mg memantine), or control (C; matching placebo), balancing the order of selection. Subjects were titrated from 5 mg medication or placebo tablets each morning with increments of 5 mg every week to reach 10 mg tablets twice per day at the third week. Subjects and all study staff, except the pharmacist were blind to treatment group.

2 Subjects were already on donepezil at time of entry into the study.

3 The Mini Mental State Exam.

4 Alzheimer’s Disease Assessment Scale: Cognitive portion, ADAS-Cog [10].

5 Activities of Daily Living, ADL.

6 NAA/Cr ratio: Scans were obtained on a 3T GE Signa Excite magnet at the Lucas Center of Stanford University. Magnetic resonance spectra were obtained from three voxels, each 2 × 2 × 2 cm, with GE scanning sequence. See Fig. 1 for detail on anatomical location of left inferior parietal lobe reported in this study. Magnetic resonance spectra were initially processed using the GE Signa Excite software, which provided peak measurements for each chemical, including NAA (n-acetyl aspartate), Cr (creatine), and MI (myo-inositol). MRS is able to measure neurochemicals, such as NAA and Cr, and it is this NAA/Cr ratio that has been found to be an indicator of degree of loss of neural tissue in AD [5].

7 Three subjects could not tolerate the study medication; two discontinued the study pills entirely and the third took a reduced dose but pill-count monitoring showed subject remained non-compliant with protocol.

Statistical analysis

The primary efficacy measure was the change from the baseline to the final study visit in the MRS NAA/Cr ratio of the inferior parietal region. The secondary efficacy measure was change from the baseline to the final study visit in the ADAS-cog [10] measure. Upon completion of the final visit of the last subject and prior to breaking the blind, analyses were conducted on the MRS measures with respect to cognitive/functional measures both in the 13 baseline and 10 final visit scans (see Table 2). Follow-up analyses were done on the 10 patients who completed the study.

RESULTS

Table 1 shows the mean and standard deviations in treatment (n = 4) and placebo (n = 6) patients at base-
MRS scans were performed on a 3 Tesla GE Signa Excite magnet. Volumes were 2x2x2 cm, with centers chosen on a plane 1 cm superior to the plane through the anterior-posterior commissure line, and to include as much gray matter as possible and as little CSF as possible. Three volumes were selected in the left hemisphere, an inferior parietal voxel, placed as far laterally as possible, behind the sylvian sulcus (shown), an occipital voxel, as posterior as possible, just lateral to the inter-hemispheric sulcus, and a posterior-cingulate voxel, anterior to the occipital cortex and behind the corpus callosum, just lateral to the inter-hemispheric sulcus.

line and follow-up for both primary and secondary outcome measures. First, the placebo and treatment groups’ inferior parietal region NAA/Cr ratio did not differ significantly at baseline (treatment group $M = 1.47 \pm 0.11$; placebo group $M = 1.38 \pm 0.10$; $p > .1$) or follow-up (treatment group $M = 1.62 \pm 0.14$; placebo group $M = 1.41 \pm 0.10$; $p = .09$). Second, the two groups’ ADAS-cog scores also did not differ significantly at baseline (treatment group $M = 44.67 \pm 10.30$; placebo group $M = 49.17 \pm 8.37$; $p > .1$) or follow-up (treatment group $M = 45.75 \pm 7.99$; placebo group $M = 50.39 \pm 8.88$; $p > .1$). As shown in Table 2, baseline NAA/Cr ratio correlated only with verbal fluency (animal naming in one minute) and age. Baseline ADAS-cog, while not correlated with age or baseline NAA/Cr ratio, did show a robust correlation with the Mini-Mental State Exam (MMSE), MMSE-extended [11], verbal fluency, and Activities of Daily Living (ADL) measures.

Over the one year treatment course, change in NAA/Cr ratios was significantly correlated with change in ADAS-cog scores ($p < 0.001$) in all patients that completed the follow-up study ($n = 10$). However, there was no significant benefit for the group treated with memantine relative to the group treated with placebo with respect to either the NAA/Cr ratios or ADAS-cog scores. The change in the memantine group was not significantly different from the change in the placebo group ($p = .09$).

**DISCUSSION**

This pilot study failed to demonstrate a benefit of memantine on the primary outcome measure, the inferior parietal NAA/Cr ratio, thus not supporting the hypothesis that memantine protects a region of the brain significantly affected by AD pathology. The secondary cognitive and functional measures also did not provide any evidence for benefit from the memantine

<table>
<thead>
<tr>
<th>NAA/Cr</th>
<th>ADAS-cog</th>
<th>MMSE</th>
<th>MMSE-extended</th>
<th>Verbal fluency</th>
<th>ADAS-cog recall</th>
<th>ADL</th>
<th>Age</th>
<th>Education</th>
<th>NAA/Cr change</th>
<th>ADAS-cog change</th>
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<tbody>
<tr>
<td>1.00</td>
<td>0.33</td>
<td>0.44</td>
<td>0.48</td>
<td>0.57*</td>
<td>0.07</td>
<td>0.46</td>
<td>0.71**</td>
<td>0.10</td>
<td>0.36</td>
<td>0.04</td>
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<tr>
<td>ADAS-cog</td>
<td>1.00</td>
<td>0.73**</td>
<td>0.62**</td>
<td>0.066*</td>
<td>0.54</td>
<td>0.59*</td>
<td>0.46</td>
<td>0.46</td>
<td>0.06</td>
<td>0.10</td>
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<tr>
<td>MMSE</td>
<td>1.00</td>
<td>0.92**</td>
<td>0.30</td>
<td>0.46</td>
<td>0.38</td>
<td>0.23</td>
<td>0.22</td>
<td>0.18</td>
<td>0.30</td>
<td>0.37</td>
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<tr>
<td>MMSE-extended</td>
<td>1.00</td>
<td>0.48</td>
<td>0.62*</td>
<td>0.51</td>
<td>0.36</td>
<td>0.21</td>
<td>0.33</td>
<td>0.37</td>
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<tr>
<td>FAS</td>
<td>1.00</td>
<td>0.29</td>
<td>0.53</td>
<td>0.60*</td>
<td>0.12</td>
<td>0.11</td>
<td>0.05</td>
<td>0.03</td>
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<tr>
<td>ADAS-cog recall</td>
<td>1.00</td>
<td>0.59</td>
<td>0.15</td>
<td>0.16</td>
<td>0.34</td>
<td>0.42</td>
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<tr>
<td>ADL</td>
<td>1.00</td>
<td>0.11</td>
<td>0.14</td>
<td>0.02</td>
<td>1.00</td>
<td>0.50</td>
<td>0.32</td>
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<tr>
<td>Age</td>
<td>1.00</td>
<td>0.08</td>
<td>0.09</td>
<td>0.20</td>
<td>1.00</td>
<td>0.74**</td>
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<tr>
<td>Education</td>
<td>1.00</td>
<td>0.08</td>
<td>1.00</td>
<td>0.02</td>
<td>1.00</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

$^* p < 0.05$ $^** p < 0.01$

1The change in NAA/Cr ratio was calculated as the mean difference between baseline and follow-up.
2The change in ADAS-cog measure was calculated as the mean difference between baseline and follow-up.
intervention in this mild dementia group. However, there was a trend towards a benefit approaching significance, so a positive effect cannot be ruled out.

In this group of patients, the NAA/Cr ratio did correspond with one functional measure (verbal fluency), and its change over time corresponded to the change in the ADAS-Cog. These observations confirm that the measurements of the NAA/Cr ratio in this study did reflect the functional changes over time associated with these AD patients.

There are several possible reasons for the lack of treatment effect of memantine in this study. First, mildly demented patients may be at a stage of the disease that while devastating to the medial temporal lobe, is only beginning to manifest adverse effects in the posterior temporal and inferior parietal convexities of the cortex [12]. Therefore, at this stage of the disease, memantine’s benefit may not be observable in the inferior parietal cortex or on measures of general cognition. MRS analysis of medial cortical structures, such as the hippocampus [13], may show benefit from memantine treatment. Second, more specific and sensitive measures of episodic memory dysfunction, the type of memory selectively affected early in AD [14], might be required to demonstrate the benefits of memantine on cognitive and functional measures. Finally, memantine is able to improve cognitive and behavioral function in moderately and severely demented patients by modulating noise at the NMDA receptor, which is severely dysfunctional later in the disease. Because the NMDA receptor might not be substantially impacted at the mild AD stage, it is possible that memantine does not exert influence in slowing down the underlying disease process earlier in the course of the disease.

The results from the present study may have shown an effect of memantine if there was a greater number of patients. However, a larger sample similar in dementia severity range to the current study [6] did not find any significant NAA/Cr ratio differences in memantine and donepezil groups over a six month period. Also consistent with results of the current study, the change in the NAA/Cr ratio was significantly correlated with the change in the ADAS-cog measure.

Recent studies have used sophisticated statistical and neuroimaging methods to enhance the sensitivity and specificity of MRS in diagnosing early AD [15]. Examination of effects with respect to APOE genotype and specificity of MRS in diagnosing early AD [15]. Examination of effects with respect to APOE genotype may resolve specific population responses to treatment [16]. It is essential to continue the exploration of treatment options in studies utilizing biomarkers as well as cognitive and functional assessments targeting the early disease process in mild AD population to establish the biological effects of interventions against AD.

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**DISCLOSURES**

Dr. Yesavage received an honorarium from Forest Research Institute prior to the proposal of this study. He has no other financial conflicts.

None of the other authors have or have had any financial involvement with Forest Companies.

Between 2003 and 2007, Dr. Ashford received honoraria from Janssen Pharmaceuticals for giving talks supporting the use of galantamine for the treatment of Alzheimer’s disease.

None of the other authors have any disclosures to make.

**REFERENCES**


