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Effects of Memantine on Cerebrospinal Fluid Biomarkers of Neurofibrillary Pathology

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Abstract

Previous studies showed that memantine inhibits tau hyperphosphorylation *in vitro*. In this study, phosphorylated tau (P-tau) and total tau (T-tau) were measured before and after 6 month treatment with memantine in 12 subjects ranging from normal cognition with subjective memory complaints, through mild cognitive impairment to mild Alzheimer's disease. Thirteen non-treated individuals served as controls. Treatment was associated with a reduction of P-tau in subjects with normal cognition. No treatment effects were seen among impaired individuals, suggesting that longer treatment time may be necessary to achieve biomarker effect in this group.

Keywords

Alzheimer's disease; biomarkers; cerebrospinal fluid; memantine; phosphorylated tau; total tau

INTRODUCTION

Glutamate excitotoxicity (overstimulation of NM-DAR N-methyl-D-aspartic acid receptors) plays an important role in the pathophysiology of Alzheimer's disease (AD). Binding of glutamate to NMDA receptors opens a cation channel resulting in calcium influx [1]. When excessive, calcium influx activates multiple pathways, ultimately leading to cell injury and death [2]. Recent studies suggest that abnormal phosphorylation of tau protein is initiated by NMDAR activation [3]. An NMDAR antagonist, memantine, widely used for treatment of AD [4], blocks receptor-associated calcium channel [1], and inhibits tau hyperphosphorylation in experimental models [5,6].

AD pathology is reflected by increases in total tau (T-tau) and phosphorylated tau (P-tau) in the cerebrospinal fluid (CSF) [7]. Despite the wide use of memantine, there is limited *in vivo* information about its effects on T-tau and P-tau using CSF biomarkers. To date, one report

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described a reduction in P-tau after 12 months of memantine treatment in patients with advanced AD [8]. We report on the effects of 6 month treatment on CSF T-tau and P-tau levels.

MATERIALS AND METHODS

Subjects

Fifteen subjects (mean age 72.5 ± 2.9 , 60% women) were recruited from the Center for Brain Health (CBH), New York University School of Medicine, to participate in an open label 6 month study. The group included 5 cognitively normal individuals (NL), 4 mild cognitive impairment (MCI) patients, and 6 patients with mild AD. Both MCI and AD are further referred to as cognitively impaired (CI). Three subjects did not complete the protocol. The reasons were: extrasystolia in one NL subject and exacerbation of behavioral symptoms in 2 AD patients. The final treated group consisted of 12 individuals (4 NL, 8 CI). Thirteen subjects (6 NL, 7 CI: 6 MCI, 1 AD) were retrospectively selected as controls from concurrent longitudinal CSF studies. All participants gave their written informed consent to NYU Institutional Review Board approved protocols. All subjects received baseline and follow-up clinical assessments (general medical, neurological, psychiatric, CSF, and laboratory examinations). At baseline, a comprehensive blood work-up (complete blood count, comprehensive metabolic, thyroid hormones level, vitamin B₁₂, lipid profile, urinalysis) and an MRI (T1, T2, FLAIR) supplemented the diagnostic work-up. Only patients who did not show brain pathology (tumor, recent stroke, hydrocephalus) or laboratory abnormalities indicating other conditions underlying cognitive decline, were included. All participants received a Global Deterioration Scale (GDS) rating [9], Brief Cognitive Rating Scale (BCRS) [10], and the Mini Mental State Examination (MMSE) [11]. The diagnosis was established at a consensus conference meeting. GDS 1 and 2 patients are cognitively normal with GDS = 1 indicating no subjective memory complaint, and GDS = 2 indicating complaint of memory change in comparison with prior adult capacity, without objective impairment on clinical evaluation [9]. All but 1 NL in our study presented with subjective memory complaints (GDS = 2). MCI was defined as GDS rating of 3, global functioning in the no-dementia range, memory complaints, and evidence of cognitive impairment on clinical interviews [9]. AD patients showed progressive impairments in multiple areas of cognition, impaired activities of daily living, met NINCDS-ADRDA [12] and DSM IV criteria [13] for AD, and in this project, had a GDS = 4 or 5.

Study design

Memantine treatment began with 5 mg once daily and gradually increased over four weeks to 20 mg (10 mg twice a day). The 20 mg dose was maintained for the remaining five months of the study. Treatment-related biomarker changes were examined between treated and non-treated groups and separately for the NL ($n = 10$) and cognitively impaired (CI) subjects ($n = 15$). Among the NL subjects, 4 were treated and 6 not treated. For the CI, 8 were treated and 7 not treated. Stable doses of other AD medications were allowed in the study: 6 treated subjects were taking Aricept.

Lumbar puncture, CSF collection, and examination

Fifteen ml of CSF was collected into polypropylene tubes using a fine 25G LP needle guided by fluoroscopy. All CSF samples were kept on ice for a maximum of 1 hour, until centrifuged for 10 min at 1500 g at 4 °C. Samples were aliquoted to 0.25 ml and were stored in polypropylene tubes at -80°C. All samples were analyzed simultaneously in one batch, blind to the treatment status.

CSF total tau (T-tau), phospho-tau 181 (P-tau₁₈₁)

CSF T-tau and P-tau phosphorylated at threonine 181 (P-tau₁₈₁) were determined using the commercially available INNOTEST hTAU Antigen kit from Inno-genetics [14]. This is a widely used tool for the determination of these biomarkers in CSF. For T-tau, the detection limit is 60 pg/ml and the coefficients of variability are 5.5% (intra-assay) and 11.6% (inter-assay). For P-tau₁₈₁, the detection limit is 15.6 pg/mL and the intra- and inter-assay coefficients of variability are <10%.

Statistical analysis

For every participant, the rates of change in biomarkers levels were calculated as: $\text{Biom}_{\text{follow-up}} - \text{Biom}_{\text{baseline}} / \text{time between exams in months}$. Between groups comparisons were conducted using U Mann-Whitney, Chi square (χ^2) or Fisher exact tests and Kruskal-Wallis ANOVA. A $p \leq 0.05$ was considered significant. *Post hoc* analyses were carried out with U Mann-Whitney test with Bonferroni correction for multiple comparisons (cutoff p value of 0.017).

RESULTS

General description

The treated and non-treated groups did not differ in age ($Z = -0.38$, $p = 0.7$), education ($Z = -0.45$, $p = 0.7$), gender distribution ($\chi^2 = 0.34$, $p = 0.6$), MMSE scores ($Z = -1.3$, $p = 0.2$), percentage of cognitively impaired participants ($\chi^2 = 0.43$, $p = 0.7$), baseline T-tau ($Z = -40.98$, $p = 0.3$), or P-tau concentration ($Z = -0.74$, $p = 0.5$). The non-treated group had a longer time between the baseline and the follow-up CSF collections ($Z = -3.4$, $p = 0.001$) (Table 1). Comparisons between treated and non-treated groups carried out separately within NL and CI yielded the same results as with the combined group, except for the time between CSF collections; it was no longer different between treated and non-treated normal subjects. Within the CI group the percentage of AD and MCI did not differ between treated and non-treated subjects ($\chi^2 = 2.1$, $p = 0.3$).

In the whole examined group ($n = 25$), biomarkers concentrations differed between NL, MCI, and AD (T-tau: Kruskal-Wallis, $\chi^2[2] = 11.64$, $p = 0.003$; P-tau: Kruskal-Wallis $\chi^2[2] = 10.12$, $p = 0.006$). For NL, MCI, and AD, the T-tau values were: 318.0 ± 63.1 , 278.0 ± 104.8 and 501.8 ± 31.0 pg/mL; P-tau values were: 55.6 ± 6.9 , 46.2 ± 11.8 , 67.0 ± 10.3 pg/mL, respectively. AD patients had higher T-tau than NL ($Z = -43.1$, $p = 0.001$) and MCI ($Z = -2.6$, $p = 0.008$) subjects. P-tau was higher in AD than in MCI ($Z = -2.7$, $p = 0.005$). No differences were found between NL and MCI.

Treatment effects

Whole group—When rates of change were compared, there was a trend-level reduction in T-tau in the treated group (-1.3 ± 4.2 versus 1.0 ± 3.5 ; $Z = -1.7$, $p = 0.09$). No effect was found for P-tau.

Cognitively normal subjects—A rate of change in P-tau was reduced in the treated group as compared to the non-treated (-0.43 ± 0.65 versus 0.16 ± 0.13 ; $Z = -2.3$, $p = 0.02$). For T-tau a trend was found (-1.43 ± 0.93 versus 0.17 ± 4.39 ; $Z = -1.5$, $p = 0.17$).

Cognitively impaired subjects—No significant treatment effects were found for either biomarker. Figure 1 represents changes in biomarkers in all study groups.

DISCUSSION

In normal subjects with subjective memory complaints, treatment with memantine was associated with P-tau decrease. No effects were seen in the impaired subjects. These findings may be consistent with the ability of memantine to inhibit abnormal tau phosphorylation in cell cultures [5]. Whether memantine treatment may have disease-modifying effects in population at risk (subjective memory complaints [15]) needs to be confirmed in larger studies. We failed to see significant memantine effects on biomarkers in the impaired subjects. This contradicts previously reported reduction in P-tau in AD patients [8]. Differences in design may be responsible for this discrepancy. In our study, the treatment lasted 6 months, in the previous study it was one year. We suspect that in impaired subjects, the treatment time necessary to achieve biomarker effects may be longer than we examined, and possibly no effects can be detected under 12 months of treatment. Contrary to this other report, where only a treated group was described, our results were compared to a control group. Given biomarkers increases observed in the treated CI subjects, in spite of the treatment, we cannot exclude more advanced pathology in this subgroup. Indeed, although not statistically significant, the number of AD patients was higher in the treated group. Our results also suggest that clinical benefit observed after 28 weeks of memantine treatment [16] may be mediated through other mechanism than reductions in tau phosphorylation.

Memantine may be more effective at blocking the high levels of receptor activity and relatively ineffective with low receptor activity [17]. Thus it is possible that cognitively normal responders with subjective memory complaints may already have substantial degree of NMDA over-activation. The observation that subjective memory complaints are a risk factor for cognitive deterioration [15] concurs with this theory. Also, energy deficits, resulting in lower ATP and membrane depolarization, may render neurons more vulnerable to normal levels of glutamate, by removing the magnesium blockade of NMDA receptor [2]. In fact, a recent study indicates that glucose metabolic rates are reduced in normal individuals with subjective memory complaints [18], supporting the hypothesis of early energy deficit.

Our conclusions are somewhat limited by the retrospective selection of the control group and differences in time to follow-up in CI subjects. We believe, however, that using rates of change minimized this bias. Also our exploratory study was small and not adequately powered to detect changes in the NL and CI subgroups.

Despite these shortcomings, the results suggest that memantine may inhibit tau hyperphosphorylation *in vivo*. In cognitively impaired individuals, the biomarker effects (if any) may be detectable only after a long period of treatment. Confirmation with a larger sample, longer observation period, and a clear relation between CSF changes and clinical outcome are needed.

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References

1. Lipton SA. Failures and successes of NMDA receptor antagonists: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. *NeuroRx*. 2004; 1:101–110. [PubMed: 15717010]

2. Wenk GL, Parsons CG, Danysz W. Potential role of N-methyl-D-aspartate receptors as executors of neurodegeneration resulting from diverse insults: focus on memantine. *Behav Pharmacol.* 2006; 17:411–424. [PubMed: 16940762]
3. Chan SF, Sucher SJ. An NMDA receptor signaling complex with protein phosphatase 2A. *J Neurosci.* 2001; 20:7985–7992. [PubMed: 11588171]
4. Farlow MR, Miller ML, Pejovic V. Treatment options in Alzheimer's disease: maximizing benefit, managing expectations. *Dement Geriatr Cogn Disord.* 2008; 25:408–422. [PubMed: 18391487]
5. Li L, Sengupta A, Haque N, Grundke-Iqbal I, Iqbal K. Memantine inhibits and reverses the Alzheimer type abnormal hyperphosphorylation of tau and associated neurodegeneration. *FEBS Lett.* 2004; 566:261–269. [PubMed: 15147906]
6. Song MS, Rauw G, Baker GB, Kar S. Memantine protects rat cortical cultured neurons against b-amyloid-induced toxicity by attenuating tau phosphorylation. *Eur J Neurosci.* 2008; 28:1989–2002. [PubMed: 19046381]
7. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet Neurol.* 2006; 368:387–403.
8. Gunnarsson DM, Kilander L, Basun H, Lannfelt L. Reduction of phosphorylated Tau during memantine treatment of Alzheimer's disease. *Dement Geriatr Cogn Disord.* 2007; 24:247–252. [PubMed: 17700020]
9. Reisberg B, Sclan SG, Franssen EH, de Leon MJ, Kluger A, Torossian CL, Shulman E, Steinberg G, Monteiro I, Mcrae T, Bocksay I, Mackell JA, Ferris SH. Clinical stages of normal aging and Alzheimer's disease: The GDS staging system. *Neurosci Res Commun.* 1993; 13 (Suppl 1):551–554.
10. Reisberg B, Schneck MK, Ferris SH, Schwartz GE, de Leon MJ. The brief cognitive rating scale (BCRS): Findings in primary degenerative dementia (PDD). *Psychopharmacol Bull.* 1983; 19:47–50.
11. Folstein, M. The Mini-Mental State Examination. In: Crook, T.; Ferris, SH.; Bartus, R., editors. *Assessment in Geriatric Psychopharmacology.* New Canaan: Mark Powley Associates; 1983. p. 47–51.
12. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology.* 1984; 34:939–944. [PubMed: 6610841]
13. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders.* 4. Washington, D.C: American Psychiatric Association; 1994.
14. Blennow K, Zetterberg H, Minthon L, Lannfelt L, Strid S, Annas P, Basun H, Andreasen N. Longitudinal stability of CSF biomarkers in Alzheimer's disease. *Neurosci Lett.* 2007; 419:18–22. [PubMed: 17482358]
15. Geerlings MI, Jonker C, Bouter LM, Ader HJ, Schmand B. Association between memory complaints and incident Alzheimer's disease in elderly people with normal baseline cognition. *Am J Psychiat.* 1999; 156:531–537. [PubMed: 10200730]
16. Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ. Memantine in moderate-to-severe Alzheimer's disease. *New Engl J Med.* 2003; 348:1333–1341. [PubMed: 12672860]
17. Lipton SA, Chen H-SV. Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine. *Cell Death Differ.* 2004; 11:18–20. [PubMed: 14647237]
18. Mosconi L, De Santi S, Brys M, Tsui WH, Pirraglia E, Glodzik-Sobanska L, Rich K, Switalski R, Mehta P, Pratico D, Zinkowski R, Blennow K, de Leon MJ. Hypometabolism and altered CSF markers in normal ApoE E4 carriers with subjective memory complaints. *Biol Psychiatry.* 2008; 63:609–618. [PubMed: 17720148]

Fig. 1. Rate of change in T-tau plotted against rate of change in P-tau. Rates of change in biomarkers are presented as: $\text{Biom}_{\text{follow-up}} - \text{Biom}_{\text{baseline}} / \text{time between exams in months (pg/mL per month)}$.

Table 1

Demographic characteristics and biomarkers concentrations in the treated and non-treated group.

Group	Treated (<i>n</i> = 12)	Non-treated (<i>n</i> = 13)
Age	72.4 ± 6.8	71.2 ± 10.0
Gender (% female)	50	61
Education (years)	16.5 ± 3.2	16.5 ± 1.3
MMSE	27.1 ± 3.3	28.7 ± 1.6
Cognitive impairment (%)	67	54
Time between CSF collection (months)	8.9 ± 2.2 *	20.1 ± 7.6
P-tau ₁₈₁ (baseline)	54.4 ± 12.7	53.8 ± 12.3
P-tau ₁₈₁ (follow-up)	54.7 ± 13.1	55.2 ± 12.1
T-tau (baseline)	362.3 ± 128.8	317.1 ± 98.9
T-tau (follow-up)	349.7 ± 109.1	343.4 ± 144.0

Values are given as means ± standard deviations (SD).

* Significant differences marked: $p < 0.05$.

In the treated group baseline lumbar puncture was performed within six months from the beginning of the treatment