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Short-term treatment with rivastigmine and plasma levels of Aβ peptides in Alzheimer's disease

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Abstract

Deregulation of APP metabolism is considered to be a key pathogenic event in Alzheimer's disease. Data from cell cultures indicate that the secretion of $A\beta_{1-42}$ might be inhibited by cholinesterase inhibitors, possibly via M1 receptors stimulation. Treatment with tacrine, a dual acetyl- and butyrylcholinesterase inhibitor, had no significant effect on mean plasma Aβ species concentrations. However, a correlation was observed between higher drug concentrations and lower Aβ levels that might indicate an effect on APP metabolism with an increased α-cleavage. $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were measured in the plasma of 28 AD subjects by means of a commercially available ELISA before rivastigmine treatment and at week 2 after the first dose of the drug (3 mg/day) had been administered. Treatment with rivastigmine exhibited a significant effect on mean plasma concentrations of $A\beta_{1-42}$ (mean difference 7.8±8.4, t=-4.9, pmean difference 7.8±8.4, t=-4.9, p<0.001) with a negative correlation with the patients age (Pearson's R=-0.40, p=0.035). No significant effect on plasma $A\beta_{1-40}$ was observed.

The observed increase of mean levels of plasma $A\beta_{1-42}$ after rivastigmine treatment might indicate an effect of the drug on Aβ metabolism, mobilization of $A\beta_{1-42}$ from deposits in the affected brain areas and a consecutive $A\beta_{1-42}$ brain-to-plasma efflux. The negative correlation between $A\beta_{1-42}$ plasma levels changes and age may be a sign of impairment of this process in the older patients. A large individual variation of the observed response, however, excludes drawing definite conclusions. Whether those subjects who respond to rivastigmine in terms of $A\beta_{1-42}$ plasma levels changes also respond clinically needs to be established.

Key words: *Alzheimer's disease, β-amyloid, plasma, Rivastigmine, cholinesterase inhibitors, butyrylcholinesterase.*

Introduction and study rationale

Cholinergic system damage has been implicated in the pathogenesis of memory dysfunction in Alzheimer's disease (AD) since the 70s when the loss of cortical cholinergic markers was found in AD for the first time. This repeated finding led to the hypothesis that cholinergic hypofunction contributes to the cognitive defects seen in AD (so called cholinergic hypothesis). Cholinesterase inhibitors are the only drugs used successfully in the clinic of dementia of AD. They exert a small but statistically significant effect on cognition as well as behavioral disturbances and activities of daily living in AD, but it is not known if

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they can affect the progression of the disease. Rivastigmine is a dual acetyl- and butyrylcholinesterase inhibitor approved for the treatment of cognitive dysfunction in AD. Recently, several studies have pointed out possible advantages of rivastigmine over its counterparts, donepezil and galantamine, by means of possible modification of the course of the disease. Firstly, acetylcholinesterase (AChE) activity in the CSF was significantly increased after treatment with donepezil and galantamine; the effect has not been observed in the rivastigmine-treated group [18]. Secondly, it has recently been shown that rivastigmine discontinuation does not result in as rapid cognitive decline as it is commonly observed with other inhibitors (mainly donepezil) [6]. There are also preliminary neuropathologic data suggesting that long-term rivastigmine treatment reduces amyloid plaques load in the brains of AD subjects. The latter supports an idea that cholinesterase inhibitors (particularly rivastigmine) use may provide more than merely symptomatic treatment [12].

Over the last several years, a number of reports have emerged suggesting that at least some cholinesterase inhibitors might take part in βAPP (β-Amyloid Precursor Protein) metabolism, influencing its secretion [13]. It has been also shown that the difference in the action of metrifonate, physostigmine, phenserine and tacrine on APP processing is independent of their selectivity for the cholinesterase enzymes. This is possibly due to the different targets used by cholinesterase inhibitors [14]. However, the promising results of cell-culture studies with the use of dual cholinesterase (acetyl- and butyrylocholinesterase) inhibitor tacrine have only partially been confirmed in humans. Although the treatment with tacrine had no statistically significant effect on plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ either at 2 weeks or at 6 weeks of administration compared to baseline levels, a correlation between higher drug concentrations and lower β-amyloid levels has been found [2]. The last observation has been interpreted as a possible indication of an effect on APP metabolism, with an increased α-cleavage.

Study aim

To test the possibility that the treatment with a dual cholinesterase inhibitor rivastigmine may influence the metabolism of βAPP (by means of a preferential stimulation of its α-cleavage) and, consequently, change plasma levels of Aβ peptides. Since in the earlier tacrine study a possible effect has been shown only at week 2 of treatment but not at week 6 (indicating an existence of a possible compensative mechanism) our study was planned in two phases: an immediate effect (2 weeks after introducing a minimal dose of rivastigmine of 3 mg/day) and delayed effect (2 weeks after a maximum well-tolerated dose is achieved). Here we present data from the first phase of the study, testing a hypothesis that an introduction of rivastigmine exerts an immediate (after 2 weeks) effect on plasma Aβ peptides levels.

Subjects and methods

Since in many Alzheimer's disease studies a large subject-to-subject variation of results is apparent and considered to be a direct result of patients' heterogeneity, we have decided to employ a selection procedure that may "enrich" our sample in "pure" AD subjects. Of initially screened 66 patients fulfilling NINCDS-ADRDA criteria for dementia in AD, we have excluded 38 for the following reasons: 14 have fulfilled criteria for mixed dementia in AD, 7 have fulfilled consensus criteria for dementia with Lewy bodies (DLB), 4 have fulfilled Lund-Manchester criteria for frontotemporal dementia (FTD), 9 had a history of at least 5 years alcohol and/or benzodiazepine misuse and/or dependence and 4 were classified as familial cases (FAD). We believe that after the above-described selection procedure we have a high chance that our sample consists mainly of AD subjects.

Twenty eight subjects (18 women; mean age 78.3±3.8, mean MMSE 17.2±3.6) were finally included in the study (see table I for baseline demographic

Table I. Demographic characteristics of the study population

Variable	Mean ± SD (except for gender)	Range
age (years)	78.3±3.8	69-88
gender (fraction of men)	0.36	
formal education (years)	7.8±3.0	4-16
age at onset	74.5±3.9	68-84
duration (years)	3.5±2.1	1-9
MMSE (points)	17.2±3.6	10-24

characteristics). The protocol of the study has been accepted by the Medical University of Lodz Ethics Committee and all the participants, as well as their caregivers have signed the informed consent. The decision of prescribing cholinesterase inhibitor was based on clinical grounds only.

Rivastigmine has been prescribed at the initial dose of 3 mg/day (divided in two doses, morning an bedtime) after a meal. A whole blood sample has been collected twice: before the first rivastigmine dose and at the second scheduled visit at week 2 of active treatment. Technically, a 10 ml blood sample was collected from fasting subjects in EDTA-containing recipients and cellular material was pelleted by centrifugation. Plasma was stored at -4°C for a maximum of 4 hours and then frozen in 1 ml aliquots and stored at -70°C until the measurements. The concentrations of Aβ peptides ($A\beta_{1-40}$ and $A\beta_{1-42}$) in the plasma were measured using a commercially available sandwich ELISA colorimetric assay (BioSource Intl, Inc) which has been shown to be sensitive enough (range 15.6-1000 pg/ml) to ensure an accurate result in the plasma. Even though platelets have been regarded as a primary source of circulating βAPP and Aβ, no sampling technique modification preventing the activation of platelets was applied, based on data indicating the lack of any associations between platelet activation and plasma Aβ levels measured with a similar method [17]. Similarly, we did not introduce any additional procedures in cases of hypercholesterolemia treated with statins, as the very recent observations failed to observe a correlation between statins treatment and plasma Aβ levels while using ELISA-based method [10].

Results

Initially, mean plasma levels of $A\beta_{1-40}$ were 170±43 pg/ml, $A\beta_{1-42}$ – 40±14 and the mean $A\beta_{1-40}/A\beta_{1-42}$ ratio has been calculated as 4.6±1.4. None of the values correlated with age of the subjects (as suggested recently by) [8] or any other demographic variable, including gender, years of formal education, duration of the disease or MMSE score [20]. Interestingly, a diagnosis of AD itself also did not exert any effect on the plasma Aβ peptides levels that were only changed in mild cognitive impairment subjects [20]; that observation is in accord with the opinion that plasma levels of Aβ peptides are unlikely to be biomarkers for AD [7,19].

The differences between plasma levels of Aβ peptides between baseline and visit at week 2 (two weeks of active treatment with 3 mg/day of rivastigmine) have been calculated using the paired samples T-test. Comparing to baseline a significant increase of $A\beta_{1-42}$ levels, but no change in $A\beta_{1-40}$ was observed (see table II). A calculated $A\beta_{1-40}/A\beta_{1-42}$ ratio decreased significantly after treatment.

Interestingly, the magnitude of change in the plasma levels of $A\beta_{1-42}$ or $A\beta_{1-40}/A\beta_{1-42}$ ratio was significantly correlated with age of the patients (the older the patients the smaller increase observed; Pearson's R=-0.40, p=0.035) but not with either the age at onset, disease duration or MMSE score. Surprisingly, the higher education the more substantial increase of $A\beta_{1-42}$ levels was observed. Both noted correlations need to be treated with caution, since large subject-to-subject variation is also evident.

Table II. Comparison of Aβ peptides levels (and $A\beta_{1-40}/A\beta_{1-42}$ ratio) before and after 2 weeks treatment with rivastigmine (paired samples T-test)

Variable	Paired differences				t	Sig. (2-tailed)
	Mean	Std. Deviation	95% CI of the difference			
			Lower	Upper		
$A\beta_{1-40}$	-3.6	12.3	-8.4	1.1	-1.55	.13
$A\beta_{1-42}$	-7.8	8.4	-11.1	-4.6	-4.95	<0.001
$A\beta_{1-40}/A\beta_{1-42}$ ratio	0.4	0.7	0.1	0.7	3.04	<0.005

Discussion

Despite being in use for more than a decade now, cholinesterase inhibitors influence on the natural course of AD is still a subject of debate. A possible advantage of rivastigmine in this regard has been recently proposed based on the results of several clinical and neuropathological studies [5,6,12,18]. However, it is not known whether this possible disease-modifying effect is related directly to a mechanism of action (so-called "dual inhibition", the term coined to describe the inhibiting activity on both acetyl- and butyrylcholinesterase) or to its other, pleiotropic functions. Cholinergic activation by means of both direct action on muscarinic receptors as well as cholinesterase inhibition may influence β-APP metabolism in the way that precludes production of potentially amyloidogenic Aβ peptides [16,22]. Such a mechanism might be possibly universal for all cholinesterase inhibitors while some might have another ways of action [21]. Finally, there are speculations that butyrylcholinesterase inhibition might offer additional benefits. Butyrylcholinesterase-positive neurons project specifically to the frontal cortex, and may have roles in attention, executive function, emotional memory and behaviour. Furthermore, BuChE activity progressively increases as the severity of dementia advances, while AChE activity declines [15]. It has also been shown that BuChE becomes associated with amyloid plaques at approximately the same time that the Aβ deposit assumes a compact β-pleated conformation. BuChE may therefore participate in the transformation of Aβ from an initially benign form to an eventually malignant form associated with neuronal loss and clinical dementia [9].

The results of our study provide the first evidence of a possible effect of rivastigmine, a dual cholinesterase inhibitor, treatment on plasma levels of Aβ peptides. A significant increase of $A\beta_{1-42}$ has been noted while no change in $A\beta_{1-40}$; this resulted in a significant decrease of $A\beta_{1-40}/A\beta_{1-42}$ ratio. Our result is in a strong contrast to the finding of [2] who did not show any changes in plasma Aβ peptides levels while treating AD patients with other dual cholinesterase inhibitor, tacrine. However, they were able to show a correlation between a higher tacrine concentration and a lower total Aβ levels after 2 weeks of treatment. This finding has been interpreted as an indication of a transitory effect on β-APP metabolism with increased β-secretase [2]. One possible explanation of the difference between the results of tacrine study and the present study with rivastigmine is that the latter inhibits BuChE relatively stronger. Therefore, in contrast to tacrine, an effect of rivastigmine might be dose-independent.

Apart from likely elucidations of how rivastigmine may specifically influence Aβ levels in plasma it is also important to discuss the source of these peptides in plasma. The very attractive option is the peptides are excreted via blood-brain barrier during cholinesterase inhibitors treatment; this would point directly to the potential disease-modifying effect. However, it is also likely that the main source of plasma Aβ are platelets. The effect of cholinesterase inhibition on β-APP metabolism *in vivo*, using platelets as a peripheral model, has been evaluated and influence on both APP trafficking and activities of its major catabolic enzymes, α- and β-secretase suggested [3,23]. Since in the present study the source of Aβ peptides has not been assessed, it is not possible to exclude the possibility that the observed effect is, at least in part, related to a peripheral cholinergic activation and not to a central action of the drug. The observed inverse correlation between age of subjects and magnitude of Aβ levels change in plasma is in agreement with the prediction of progressive, age-related damage of the blood-brain barrier [11] as well as the relevant animal data [1]. It may also indicate that rivastigmine might act better in younger subjects. Indeed, this has recently been shown in the long-term head-to-head study comparing rivastigmine with donepezil. In this study patients younger than 75 years tend to respond better to rivastigmine than to an active comparator, donepezil [4].

Finally, it is important to discuss strengths and shortcomings of the present study. The number of subjects included is relatively small and the observation time short. One cannot predict whether the observed effect is transient, that would favor peripheral action of the drug as a possible explanation or more stable over the time. Phase two of the study (the assessment of Aβ plasma levels after a longer period of time and relationships to clinical treatment response) would address this issue. In addition, rivastigmine was the only one inhibitor used that precludes a direct comparison of inhibitors effects and makes uniqueness of

rivastigmine action on plasma Aβ almost purely speculative. The strong point of the study is the subjects selection. The method used makes the cohort as homogenous as it is possible on clinical grounds only. One cannot, naturally, exclude the chance that in our sample there were patients suffering from other than AD disorders, it seems to be, however, quite unlikely.

To conclude, the results of the present study suggest that short-term treatment of rivastigmine influences the plasma levels of Aβ peptides in the way that restores the balance between the mutually exclusive α- and β-secretase pathways in APP processing and promotes a non-amyloidogenic processing pathway. Alternatively, there is also a possibility that the change in Aβ plasma levels represents a marker of treatment response; to test this hypothesis, longitudinal data from the same set of subjects shall be used.

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