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Cerebrospinal fluid levels of biomarkers and activity of acetylcholinesterase (AChE) and butyrylcholinesterase in AD patients before and after treatment with different AChE inhibitors

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Abstract In order to evaluate the biochemical effects of long-term treatment with inhibitors of acetylcholinesterase (AChE) in patients with Alzheimer's disease (AD), we measured the activities of AChE and butyrylcholinesterase (BuChe) and the concentrations of β-amyloid (1–42), τ and phosphorylated τ proteins in the cerebrospinal fluid (CSF). A total of 91 patients suffering from probable AD of mild to moderate degree were treated for 6 months with donepezil (n=59), galantamine (n=15), rivastigmine (n=10), or placebo (n=7). AChE activity in CSF was significantly increased after treatment with donepezil and galantamine; the opposite was observed in the rivastigmine-treated group. Untreated patients did not show any AChE activity variation. BuChE did not show any change in any of the groups studied. Mean values of β-amyloid(1–42), total τ and phosphorylated τ also did not vary significantly. We conclude that AChE inhibitors induce different effects on CSF AChE activity, while other CSF biomarkers are not significantly affected by treatment.

Acetylcholinesterase (AChE) colocalizes with β-amyloid in neuritic plaques and accelerates the assembly of amyloid β-peptides into fibrils deposited in the brain of patients with Alzheimer's disease (AD). Conversely, the β-amyloid protein regulates AChE expression, assembly and glycosylation in cell cultures, transgenic mice and Alzheimer brain, thus creating a vicious circle leading to an increased accumulation of β-amyloid. AChE inhibitors have been suggested to enhance the release of nonamyloidogenic soluble derivatives of amyloid precursor proteins (APPs) in vitro and in vivo and possibly to slow down formation of amyloidogenic compounds in brain [1–3]. Therefore, inhibition of AChE activity might influence APP processing and β-amyloid deposition.

With respect to the potential influence of AChE inhibitors on τ protein, there is a recent in vitro study showing that these drugs are able to modulate phosphorylation and levels of τ protein in SH-SY5Y cells through an interaction with nicotinic receptors [4]. According to these premises, the biochemical effects of AChE inhibitors can be monitored by means of biological markers in the cerebrospinal fluid (CSF), e.g. AChE and butyrylcholinesterase (BuChE) activities and the concentrations of β-amyloid and total and phosphorylated τ proteins. Therefore, we carried out an explorative study by measuring activities of AChE and BuChE and concentrations of β-amyloid(1–42), τ and phospho-τ proteins in CSF of AD patients before and after long-term treatment with different AChE inhibitors.

We studied 91 patients suffering from probable AD of mild to moderate degree, recruited in Malmö and Piteå, Sweden and in Perugia, Italy. A total of 84 subjects were treated with one of three AChE inhibitors for 6 months: 59 patients received donepezil, 15 were treated with galantamine, and 10 received rivastigmine. As control group, 7 AD patients enrolled in a previous double-blind placebo controlled clinical trial who underwent lumbar puncture before and 6 months after treatment with placebo were included. AChE and BuChE activities in the CSF were measured spectrophotometrically. β-Amyloid(1–42), τ and phospo-τ [5] were determined using a specifically constructed sandwich ELISA (Innogenetics, Ghent, Belgium).

All the patients (or their nearest relatives) gave informed consent to participating in the study. At the time of enrollment in the study, none of the patients were being treated with any drug interfering with cognitive functions. For statistical analysis, normal distributions were tested using the Shapiro-Wilk test; if normality was rejected, non-parametric tests were employed. Subgroup comparisons were done using the *t* test, after Bonferroni correction for multiple comparisons.

Treatment with donepezil caused a significant and dose-related increase of CSF AChE activity as opposite to completely unmodified BuChE activity. None of the other CSF markers (β-amyloid(1–42), total τ, phospho-τ) showed a significant change after treatment.

Similarly to that observed for donepezil, treatment with galantamine caused a significant increase of AChE activity in CSF, while BuChE activity remained unchanged. No variations were observed in the concentrations of the other biochemical markers.

Although only 10 patients were treated with rivastigmine, a significant reduction of AChE activity was documented in this group. BuChE activity and levels of the other biomarkers did not show major variations.

No significant variations were observed in any of the biochemical parameters studied in the 7 control subjects.

With respect to the effect on AChE activity, the drugs tested behaved differently: donepezil and galantamine caused a marked (donepezil>galantamine) increase; rivastigmine induced a significant decrease. These findings are in agreement with two recent reports [6, 7]. The different mechanisms of action of these drugs might explain this result: donepezil and galantamine are reversible inhibitors, while rivastigmine is a pseudo-irreversible inhibitor, implying that in the process of inactivating AChE a cleavage of the parent molecule takes place. Donepezil caused a strong and dose-dependent up-regulation of the enzyme activity, probably due to its non-competitive (i.e. non-compensatory) action; galantamine has a competitive action, which does not depend on the absolute concentration of the drug but more on the relationship with the substrate concentration. None of the drugs tested influenced BuChE activity. This was expected for donepezil and, to a less extent, also for rivastigmine [8], while, to our knowledge, no data are available for galantamine relative to human CSF studies. The other CSF biomarkers for AD - β-amyloid(1–42), τ and phospho-τ - did not show any significant change after treatment.

In conclusion, this study showed that: (i) AChE inhibitors induced different effects on AChE activity in the CSF and, at least for donepezil, the effect was dose-dependent; (ii) the biochemical effects of these drugs were detected in CSF and different treatments were distinguished; (iii) other CSF biomarkers of AD were not significantly affected by treatment with AChE inhibitors. The possibility to detect in CSF the biochemical processes taking place in the central nervous system of AD patients treated with anti-dementia drugs confirms the importance of this approach for a better knowledge of the pathophysiology of the disease and will allow us to demonstrate the actual impact of the new therapeutic strategies (e.g. anti-β-secretase drugs, anti-amyloid vaccine) aimed at interfering with the pathogenetic events of the disease.

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