SHORT REPORT

Cerebrospinal fluid acetylcholinesterase in progressive supranuclear palsy: reduced activity relative to normal subjects and lack of inhibition by oral physostigmine

John R Atack, Irene Litvan, Leon J Thal, Conrad May, Stanley I Rapoport, Thomas N Chase

Abstract

Acetylcholinesterase (AChE) activity was measured in lumbar cerebrospinal fluid (CSF) of 11 patients with progressive supranuclear palsy (PSP) and 18 age-matched healthy control subjects. Mean CSF AChE activity in PSP subjects was significantly reduced by 31% relative to control subjects ($p < 0.002$). In the light of evidence of a central cholinergic deficit, physostigmine was administered orally (0.5–2.0 mg every two hours, six times a day for 10 days) to eight of the 11 PSP patients. CSF was sampled when the patients were on placebo and when receiving physostigmine and CSF AChE and butyrylcholinesterase (BChE) activities were measured. There was no significant change in either CSF AChE or BChE activities following physostigmine treatment. These data suggest that the doses of physostigmine used were insufficient to produce marked inhibition of AChE within the central nervous system.

Progressive supranuclear palsy (PSP) is a chronic, progressive disorder characterised clinically by extrapyramidal rigidity, bradykinesia, pseudobulbar palsy, supranuclear ophthalmoplegia and dementia.¹ Neuropathologically, there is neuronal degeneration and neurofibrillary tangles (which are distinct from those seen in Alzheimer's disease) in the pontine tegmentum, dentate nucleus, red nucleus, substantia nigra, subthalamic nucleus and globus pallidus.¹ Neurochemically, there is degeneration of the nigrostriatal dopaminergic system, similar to that seen in Parkinson's disease, which is thought to result in the Parkinsonian extrapyramidal features of PSP.^{1,2} However, and in contrast to Parkinson's disease, the mesolimbic and mesocortical dopaminergic projection systems seem to be relatively spared in PSP.^{1,2}

So far as the central cholinergic systems are concerned, there is a loss of large, probably cholinergic³ neurons in the striatum⁴ and reduced levels of striatal choline acetyltrans-

ferase activity have been reported.⁵ Degenerative changes are also seen in the cholinergic basal forebrain,^{5,6} although alterations in cortical choline acetyltransferase activity^{5,7} and nicotinic receptor binding⁸ are more variable and less severe than those seen in Alzheimer's disease.⁹ In addition, there is a marked degeneration of the hindbrain pedunculopontine tegmental nucleus pars compacta,¹⁰ a putative cholinergic nucleus which projects to many nuclei of the extrapyramidal system.¹¹

The purpose of this study was to determine whether reported postmortem deficits in central cholinergic systems in PSP are reflected *in vivo* by changes in cerebrospinal fluid (CSF) acetylcholinesterase (AChE) activity. Furthermore, PSP subjects were also given oral physostigmine in a double-blind cross-over study and, as an index of the CNS AChE-inhibitory effects of physostigmine treatment, CSF AChE activities were measured following physostigmine treatment and were compared to activities following placebo. In addition, CSF butyrylcholinesterase (BChE) was also measured since, although this enzyme is not related to central cholinergic function and probably derives from plasma, it is nevertheless sensitive to physostigmine. Consequently, the measurement of BChE activity following physostigmine administration serves as an additional index of whether sufficient drug enters the CNS and produces cholinesterase inhibition.

Patients and methods

Subject selection

The clinical diagnosis of PSP was made in 11 subjects [five men and six women; mean (SD) age 64 (6) years] according to: 1) age at onset greater than 50 years; 2) extraocular movement abnormalities characterised by supranuclear vertical (with or without horizontal) palsy;¹² 3) Parkinsonian signs (including bradykinesia, postural or gait instability, axial dystonia and rigidity) in the absence of resting tremor; 4) pseudobulbar signs, including dysarthria and dysphagia; and 5) progressive course. The mean (SD) duration of symptoms was 35 (13) months. During

The Laboratory of
Neurosciences,
National Institute on
Aging

J R Atack
C May
S I Rapoport

Experimental
Therapeutics Branch,
National Institute of
Neurological and
Communicative
Disorders and Stroke,
National Institutes of
Health, Bethesda,
Maryland

I Litvan
T N Chase

Neurology Service,
San Diego VA Medical
Center, San Diego,
California, USA
L J Thal

Correspondence to:
Dr Atack, Merck Sharp and
Dohme, Neuroscience
Research Centre, Terlings
Park, Eastwick Road,
Harlow, Essex CM20 2QR,
UK.

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this study no subjects received centrally-acting drugs except one who continued to receive levodopa-carbidopa. Parkinsonian rigidity and akinesia was generally mild to moderate [Columbia Rating Scale (SD) 11 (4) range 5–18] and dementia was generally mild [Mattis Dementia Rating Scale, 123 (8) range 112–139; Weschler Memory Quotient, 96 (10) range 79–110]. Healthy control subjects [11 male, seven female; mean (SD) age 65 (10) years] were selected according to criteria described elsewhere.¹³

Physostigmine treatment

Physostigmine treatment was as described previously.¹⁴ Briefly, eight of the 11 PSP patients received escalating doses of physostigmine six times daily (0.5 mg per dose on day 1 rising to 2.0 mg per dose on day 4). The Buschke Selective Reminding Test¹⁵ was used to assess the best dose for each individual. Patients were subsequently randomised to a 10 day, placebo-controlled, double-blind, cross-over trial of physostigmine. Each individual received their previously determined best dose [mean (SD) 1.25 (0.46) mg/dose, range 0.5–2.0 mg/dose] six times a day at two hour intervals.

Lumbar puncture

Cerebrospinal fluid was collected on the tenth day of placebo or physostigmine treatment at approximately 8.30 am (about 30 minutes after the second morning dose). CSF was also collected at a similar time from the three PSP patients not admitted into the physostigmine study. CSF was removed from healthy normal subjects¹³ using procedures comparable to those employed for the PSP patients. CSF was immediately frozen on dry ice and kept at -70°C until assayed.

Assay procedures

Acetylcholinesterase and BChE activities were assayed as described previously.¹³ Thus 0.5 mM acetyl- β -methylthiocholine and 0.5 mM butyrylthiocholine were used as relatively specific substrates for the assay of AChE and BChE activity, respectively. The coefficient of variation of the AChE assay was 2.9% and that for BChE was 2.4%. Total protein concentrations were determined by the method of Lowry *et al.*¹⁶

In the event that physostigmine administration produced inhibition of CSF AChE and BChE activities, an indirect method was devised to estimate CSF physostigmine concentrations. Thus increasing concentrations of physostigmine were incubated with samples of CSF from three normal and three PSP subjects. The percentage activity was plotted as a function of physostigmine concentration and from these "standard curves" it was possible to predict the CSF physostigmine concentration corresponding to any given % inhibition of CSF AChE or BChE activity. CSF physostigmine concentrations were also measured directly using a modification of a high-performance liquid chromatography (HPLC) method.¹⁷ This assay has a sensitivity

of 0.05 ng/ml.

Values shown are mean (SD). Comparisons between groups were made using Student's *t* test and the level of significance was taken as $p < 0.05$.

Results

AChE activities in PSP and healthy control subjects

The figure shows AChE activity in control and PSP subjects. In PSP, CSF AChE activity was significantly reduced by 31% ($p < 0.002$) relative to control values [14.7 (5.1) and 21.3 (4.8) nmol/min/ml, respectively]. Neither BChE activity nor total protein concentrations differed significantly between groups [BChE activity = 10.0 (3.0) and 9.4 (3.6) nmol/min/ml; protein = 0.56 (0.15) and 0.55 (0.18) mg/ml in control and PSP groups, respectively].

Effect of physostigmine on AChE and BChE activities

Physostigmine treatment had no significant effect on either AChE or BChE activity in CSF of PSP patients. Following treatment, AChE activity (SD) was 98 (5%), and BChE 97 (8%) of activity observed following placebo administration.

To estimate the CSF physostigmine concentration, AChE and BChE activities were measured as a function of physostigmine concentration. The IC_{50} of physostigmine versus AChE and BChE did not differ between control and PSP CSF and was 30–40 nM for both enzymes. Given the relatively small variations in AChE and BChE activities during the 10 day period between placebo and physostigmine lumbar punctures, as well as the small coefficient of variation of the assays ($< 3\%$), we estimated that we could have reliably detected a 10% enzyme inhibition, which would correspond to a physostigmine concentration of 1–2 nM. Therefore since we could not detect any change in either AChE or BChE activities following physostigmine treatment, CSF physostigmine concentrations must have been less than 1–2 nM (0.28–0.55 ng/ml free base).

In addition, CSF physostigmine concentrations were also measured directly using HPLC.

Figure AChE activity in lumbar CSF of age-matched healthy control subjects and PSP patients. AChE activity was significantly lower (*, $p < 0.002$) in PSP patients [mean (SD) AChE activity = 14.7 (5.1) nmol/min/ml] compared with control subjects [21.3 (4.8) nmol/min/ml].

In two of the eight patients studied, physostigmine concentrations were below the limits of detection (< 0.05 ng/ml). In the other six, physostigmine concentrations ranged from 0.07–0.35 ng/ml. There was, however, no correlation between CSF physostigmine concentrations and the dose administered, that is, patients with the highest CSF physostigmine concentrations were not necessarily the patients receiving the highest oral doses (1.5 or 2.0 mg/dose). These data are in good agreement with the physostigmine concentrations estimated from the activity versus physostigmine concentration curves (less than 0.28–0.55 ng/ml).

Discussion

AChE activity in PSP and healthy control subjects

Reduced CSF AChE activity in PSP is consistent with a central cholinergic deficit in these patients. However, reduced CSF AChE activity in PSP is unlikely to be solely due to degeneration of the cholinergic basal forebrain projection. Thus in PSP the degeneration of these systems and consequent loss of cortical cholinergic markers^{5–8} is less pronounced than in Alzheimer's disease⁹ yet the reduction in CSF AChE activity in PSP is greater than that observed in Alzheimer's disease.¹³

Reduced CSF AChE activity in PSP may also be partly related to degeneration of the cholinergic pedunculopontine nucleus,¹⁰ which projects to many nuclei of the extrapyramidal system. In addition, the loss of cholinergic activity in the striatum,⁵ whether it be as a consequence of loss of putative cholinergic input from the pedunculopontine nucleus or a degeneration of intrinsic cholinergic neurons^{4,5} may also contribute to reduced lumbar CSF AChE activity in PSP. CSF AChE activity may be relatively sensitive to changes in striatal cholinergic function given the high AChE content of the striatum¹⁸ and the proximity of the striatum to CSF in the cerebral ventricles.

Effect of physostigmine on AChE and BChE activities

Since peak plasma physostigmine concentrations are achieved 20–40 minutes after oral administration^{17,19} and the time course of physostigmine in rat brain (and presumably CSF) parallels that of plasma,²⁰ we chose 30 minutes as an optimum time to sample CSF after oral administration of physostigmine to PSP patients. However, following oral administration of 0.5–2.0 mg physostigmine, no effect on CSF AChE or BChE activity was observed. In contrast, using dosing schedules similar to those employed in this study, CSF AChE inhibition as high as 70% has been reported following oral doses of 1–3.5 mg.²¹ This discrepancy may be related in part to methodological differences. Thus in this study, CSF AChE and BChE activities after physostigmine treatment were compared with activities after placebo treatment, whereas in previous studies the degree of CSF AChE inhibition was assessed using a reactivation analysis in which

CSF was incubated at 37°C for two to 28 hours.²¹

The direct measurement of CSF physostigmine concentrations by HPLC revealed CSF levels, where detectable, of between 0.07 and 0.35 ng/ml. At these physostigmine concentrations, CSF AChE activity would be inhibited by less than 10% and would therefore explain why inhibition of AChE (or BChE) could not be detected in our study. Moreover, the indirect estimation of CSF physostigmine concentration (using activity versus physostigmine concentration curves) of less than 1–2 nM (less than 0.28–0.55 ng/ml) agrees well with the physostigmine concentrations measured directly using HPLC (less than 0.35 ng/ml). This suggests that this indirect method is a useful means of estimating CSF physostigmine concentrations, not only since it measures the pharmacodynamics (% inhibition of AChE) rather than the pharmacokinetics (drug concentration) of physostigmine, but also because it is a relatively straightforward, reproducible and inexpensive means for determining CSF concentrations of physostigmine or, indeed, any other cholinesterase inhibitor, for example, tetrahydroaminoacridine (THA).

The lack of pronounced inhibition of CSF (and presumably brain) AChE by doses of 0.5–2.0 mg physostigmine may explain the relatively modest and inconsistent behavioural changes observed in these patients.¹⁴ In future, the use of controlled release physostigmine preparations, resulting in sustained plasma physostigmine levels²² or the development of novel physostigmine analogues²³ may prove to be more useful in the modulation of central cholinergic activity.

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