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Inhibition of acetyl- and butyryl-cholinesterase in the cerebrospinal fluid of patients with Alzheimer's disease by rivastigmine: correlation with cognitive benefit

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Summary. Cholinesterase (ChE) inhibition represents the most efficacious treatment approach for Alzheimer's disease (AD) to date. This multiple-dose study has examined the relationship between inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities in the cerebrospinal fluid (CSF) and cognitive change (measured by the Computerised Neuropsychological Test Battery [CNTB]) following administration of the ChE inhibitor, rivastigmine (Exelon®). In 18 patients with mild to moderate AD, CNTB scores, activities of AChE and BuChE in the CSF, and plasma BuChE activity were determined prior to treatment with rivastigmine. Doses of rivastigmine were then titrated (1 mg b.i.d./week) to final doses of 1, 2, 3, 4, 5 or 6 mg b.i.d. ($n = 3$ per dose). Following treatment with the target dose of rivastigmine for at least 3 days, CNTB scores were re-determined. CSF samples were continuously collected together with plasma samples prior to and for 12 hours after the final dose of rivastigmine, and AChE and BuChE activities determined.

AChE in CSF and BuChE in plasma were dose-dependently inhibited by rivastigmine treatment. The inhibition of BuChE in CSF was not clearly dose-dependent. A statistically significant correlation was observed between the change in CNTB summary score and inhibition of AChE activity ($r = -0.56$, $p < 0.05$) and BuChE activity ($r = -0.65$, $p < 0.01$) in CSF. Improvement in speed-, attention- and memory-related subtests of the CNTB correlated significantly with inhibition of BuChE but not AChE activity in CSF. Weak or absent correlation with change in cognitive performance was noted for inhibition of plasma BuChE. These results indicate that cognitive improvement with rivastigmine in AD is associated with central inhibition of ChEs and support a role for central BuChE in addition to AChE inhibition in modulating cholinergic function in AD.

Keywords: Alzheimer's disease, cholinesterase inhibitor, acetylcholinesterase, butyrylcholinesterase, rivastigmine, cerebrospinal fluid, cognition.

Introduction

Two different cholinesterase (ChE) enzymes are present in the human brain: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE is present at cholinergic nerve terminals (either intraneuronally, membrane bound or in the synaptic cleft) whereas BuChE is associated with glial cells or neurons (Wright et al., 1993; Mesulam and Geula, 1994). Although AChE comprises 90% of the total ChE in the temporal cortex of normal brain and mediates the inactivation of most synaptic acetylcholine (ACh) (Perry et al., 1978), there is increasing recognition that BuChE may also be involved in the hydrolysis of ACh and play an important role in Alzheimer's disease (AD). It has been proposed that differences in enzyme kinetics of AChE and BuChE result in varying efficiencies of ACh hydrolysis dependent on substrate concentration, and that the physiological role of BuChE is to hydrolyse excess ACh (Giacobini, 2000). This is supported by the observation that BuChE hydrolyses the ACh surrogate acetylthiocholine in the presence of an AChE-specific inhibitor in human brain (Mesulam et al., 2002a), and that administration of a selective BuChE inhibitor increases extracellular ACh levels in the rat cortex (Giacobini, 2000). The recent characterisation of mice in which the AChE gene was selectively inactivated, but who developed an intact cholinergic system utilising BuChE, further demonstrates that BuChE is able to hydrolyse ACh, may compensate for AChE loss, and may play an important role in cholinergic transmission (Xie et al., 2000; Mesulam et al., 2002b).

Concurrent with the decline in cholinergic function as AD progresses, there is a loss of brain AChE activity, while BuChE activity remains unchanged or even increases by 40–90% (Perry et al., 1978; Arendt et al., 1992), probably due to glial cell proliferation (Geula and Mesulam, 1994). Consequently the ratio of BuChE to AChE increases dramatically in the cortex of patients with AD from 0.6 to as high as 11 (Giacobini, 2000). The close proximity of glial cells to the synapse offers the potential for these increased levels of BuChE to regulate synaptic ACh thereby modulating cholinergic function (Giacobini, 1997).

Cerebrospinal fluid (CSF) lumbar puncture provides a safe and relatively non-invasive method by which to examine enzyme activities in the central nervous system. Interpretation of CSF-derived data is complicated however by the multiple and complex origin of proteins in this fluid. AChE in CSF originates mainly from brain tissue and spinal cord as a result of secretory processes (Bareggi and Giacobini, 1978; Scarsella et al., 1979), whereas the origin of BuChE involves several compartments: glial cells (mainly astrocytes), neurons and plasma (Scarsella et al., 1979). The ratio may be changed in AD patients, and more BuChE could originate from the central compartment due to an altered blood-brain-barrier. It is known that CSF AChE activity increases progressively with age in normal control subjects while it does not change significantly in mild to moderate AD patients followed up to

12 months (Elble et al., 1987). Other authors have reported a reduction in CSF AChE activity together with an increase in BuChE activity in AD patients (Arendt et al., 1992).

This study has investigated a possible relationship between the extent of AChE and BuChE inhibition in CSF and cognitive improvement in AD patients following treatment with rivastigmine. The activity of the individual ChEs in CSF have been measured in this study as putative markers of central AChE and BuChE activities. The relationship between inhibition of central ChE activity and plasma or red blood cell (RBC) ChE is not well defined and varies with compound and route of administration (Hallack and Giacobini, 1989, 1987). It has previously been demonstrated in healthy volunteers that rivastigmine treatment results in minimal RBC AChE inhibition compared with a significant reduction in plasma BuChE activity (Kennedy et al., 1999). To clarify this issue further, our analysis also included the measurement of plasma BuChE activity. Changes in CSF AChE and BuChE activities and plasma BuChE activities were then correlated with cognitive changes measured using the Computerised Neuropsychological Test Battery (CNTB) (Veroff et al., 1991).

Material and methods

The design and method of data collection have been presented in detail elsewhere (Cutler et al., 1998). Briefly, 18 patients (9 male, 9 female), aged between 48 and 82 years (mean 62.6 ± 10.9) who met NINCDS-ADRDA criteria for probable AD were recruited into a single-centre, open-label study. Patients were required to have a Mini-Mental State Examination (MMSE) score of 10 to 26, a modified Hachinski Ischaemia score of ≤4 and no evidence of a current significant physical illness. Female subjects were required to be of non-child bearing potential. Patients were excluded if the recent use of cholinergic or psychoactive medication was confirmed. Detailed inclusion and exclusion criteria are presented in the previous report (Cutler et al., 1998). The study was carried out in accordance with Good Clinical Practice following guidelines presented in the United States Code of Federal Regulations dealing with clinical studies, the guidelines of the European Community and the Declaration of Helsinki.

Study design

Patients were screened for eligibility and a practice CNTB session administered during a 42-day period prior to commencing the study. Patients were sequentially assigned to one of six treatment groups (n = 3 per group). One day prior to commencing study treatment, patients were admitted to the study centre and baseline assessments performed. A venous blood sample and a 2 ml clear CSF sample (lumbar puncture) were obtained for the measurement of enzyme activities, and baseline CNTB scores were determined. Patients remained in hospital overnight and the following morning were administered the first dose of rivastigmine. Patients were discharged with 1 weeks' supply of study medication, to be taken twice daily. Doses were titrated in 1 mg b.i.d./week increments to attain final target doses of 1, 2, 3, 4, 5 or 6 mg b.i.d. The groups were ordered from highest dose level to lowest so that patients not tolerating their designated dosage regimen could reduce dose and be evaluated as part of a group with a lower dosage regimen that had not yet included three patients. Patients returned to the hospital weekly for tolerability assessments and dispensing of study medication.

Patients who had tolerated their target dose for at least 3 days, or a better tolerated lower dose for at least 7 days, were re-admitted to hospital for re-assessment of CNTB scores and plasma and CSF enzyme activities. The CNTB was administered in an

afternoon and patients then received the evening dose of study medication prior to an overnight stay at the hospital. The following morning, patients were catheterised for continuous CSF sampling as detailed previously (Cutler et al., 1998). Samples of CSF (1.2 ml/12 minutes) were obtained 0.5 hours before and for up to 12 hours following administration of the final dose of study medication. Samples were placed on dry ice and subsequently stored at −20°C pending analysis. Venous blood samples were also obtained 0.5 hours before and for up to 24 hours post-dose. Following separation of plasma by centrifugation (at 4°C) samples were placed on dry ice and also stored at −20°C. Following completion of CSF sampling, patients remained in hospital for at least 24 hours or until able to ambulate.

Determination of CNTB score

The CNTB is comprised of 11 subtests evaluating a variety of memory, attentional and reaction time tasks (Veroff et al., 1991). It is sensitive to a broad range of cognitive performance and has wide application in diverse patient populations. The subtests are known to be sensitive to the changes in psychological functioning associated with AD and thus the CNTB has been reported as valid and reliable for assessment of this patient group (Veroff et al., 1991; Cutler et al., 1994; Veroff et al., 1998). Stimulus presentation and data collection are computer-controlled, which maximises standardisation and enables simple test administration. The battery of tests is presented in a standard order.

The CNTB subtests include Boston Naming Test-short form, paired associate learning with selective reminding, paired associate learning with selective reminding/delayed recall, visual matching, visual matching/delayed recall, visual memory, word list learning with selective reminding, word list learning with selective reminding/delayed recall, finger tapping (both, left, right), choice reaction time (time, percent correct) and simple reaction time. With the exception of finger tapping and choice reaction time (time), tasks are rated as a percentage correct and the mean score from these provides a summary score. Consequently deterioration of a patient's cognitive function is seen as a decrease in summary score and an increase in reaction times.

The results from the CNTB were analysed as absolute change from the baseline scores attained prior to commencing study medication. The 11 CNTB subtests normally provide 14 outcome parameters. However, inspection of the data showed that 100% correct answers were provided by more than 50% of the patients in three of the subtests (choice reaction time (percent correct), visual matching and visual matching-delayed recall), leaving little or no room for improvement on these parameters. As a consequence, these three subtests, although included in the CNTB summary score, were not considered separately.

Determination of ChE activity

Aliquots of CSF or diluted (1:50) plasma were assayed for AChE and BuChE activity according to the method of Ellman et al. (1961). For more details see Cutler et al. (1998). For the determination of AChE activity, acetylthiocholine-iodide was used as a substrate at a concentration of 0.5 mM in the presence of 10^{-4} M ISO-OMPA (tetra-isopropylpyrophosphoramide) as a selective inhibitor of BuChE and for the demonstration of BuChE activity, butyrylthiocholine-iodide was used as a substrate in the presence of 10^{-6} M 1,5-bis(4-allyldimethyl-ammoniumphenyl) pentane-3-one dibromide (BW284C51) as a selective AChE inhibitor. Enzyme activity is expressed per ml of CSF.

Enzyme activity (measured as OD units/minute) at each time point following administration of the final dose of rivastigmine was expressed as a percentage change from the value obtained immediately prior to drug administration (t_0). Expressing the enzyme activity as a percentage change from t_0 rather than as absolute differences was deemed appropriate in order to reduce the impact of high inter-individual variability in enzyme activities observed at baseline and t_0. The area-under-the-curve (AUC_{0-t}) determined by the linear trapezoid method was the time interval weighted average of a patient's enzyme

inhibition responses over time (t) till 12 hours for CSF measurements, and 12 and 24 hours for plasma. Since CSF data were available for 12 hours, only the 12 hour plasma data were considered for the current analyses.

Statistical methods

Pearson's correlation coefficient *r*, was used to assess the relationship between dose and enzyme inhibition. Three patients were found to have CSF BuChE values at t_0 which, within the series of respective patients, were clearly abnormal, i.e. lower by a factor of three to eight than the values immediately before and after the respective measurements. Two of these three subjects also had conspicuously low CSF AChE values at t_0. For statistical analysis, the t_0-values for AChE and BuChE in CSF were replaced in these three cases with the values obtained immediately before t_0, i.e. the values obtained at -0.4 hours. In addition, all correlations involving changes from t_0 in CSF AChE and BuChE were also calculated excluding these three patients with abnormally low t_0 values.

Pearson correlation coefficients were also calculated to determine the relationship between changes in CNTB summary score, the 11 CNTB outcome parameters and AChE or BuChE inhibition in the CSF or plasma. In all instances statistical significance was defined as $p < 0.05$.

Results

Inhibition of AChE and BuChE activities in plasma and CSF

Activities of AChE in CSF and of BuChE in plasma and CSF were strongly inhibited by rivastigmine (Table 1). Maximum inhibition of AChE in CSF occurred between 2 and 5 hours after administration, while maximum inhibition of BuChE, both in plasma and CSF occurred between 2 and 4 hours after administration (for details see Culter et al., 1998).

Correlations between rivastigmine dose and enzyme inhibition in CSF and plasma

The relationship between dose and enzyme inhibition in plasma and CSF are presented in Table 2. A highly significant correlation was observed between dose and BuChE inhibition in plasma over 12 hours. Correlation between dose and AChE inhibition in the CSF was also significant. Excluding the three patients with abnormal values at t_0 resulted in a similarly high correlation between dose and AChE inhibition in CSF. The correlation between dose and

Table 1. Maximum mean inhibition of AChE and BuChE activities

	Rivastigmine dose					
	1 mg b.i.d.	2 mg b.i.d.	3 mg b.i.d.	4 mg b.i.d.	5 mg b.i.d.	6 mg b.i.d.
AChE in CSF	20.0%	35.6%	46.0%	28.2%	55.6%	61.7%
BuChE in plasma	6.8%	9.8%	34.0%	17.2%	50.7%	33.9%
BuChE in CSF	23.9%	27.9%	76.6%	26.0%	50.9%	61.8%

Values shown are maximum mean changes (n = 3 per group) from values obtained immediately prior to drug administration (t_0)

Table 2. Correlations between rivastigmine dose and enzyme inhibition in CSF and plasma

	n∞	Dose	AChE inhibition† CSF	BuChE inhibition† CSF
BuChE inhibition† –	18	−0.714***	0.583**	0.447
plasma 12 hours	15	NA	0.662**	0.286
AChE inhibition† –	18	−0.694**	–	0.669**
CSF	15	−0.614*	–	0.768**
BuChE inhibition† –	18	−0.303	0.669**	–
CSF	15	−0.311	0.768**	–

∞Values presented are for n = 18 (three subjects with substituted CSF AChE and BuChE values at t_0) and for n = 15, excluding these three subjects (see text). †The inhibition of enzymes is measured as percentage difference from t_0 and these percent changes were then integrated over time. As a consequence, AUC values are negative for ChE inhibition. A negative correlation co-efficient therefore indicates that inhibition increases as dose increases. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

BuChE inhibition in CSF was lower and not statistically significant. Inspection of the data showed that three patients had very strong inhibition of BuChE in CSF at relatively low doses (one at 4 mg/day, two at 6 mg/day) which would have influenced this outcome.

Correlations between rivastigmine dose and changes in CNTB measures

The relationships between dose and changes in CNTB measures are presented in Table 3. There was a positive but not statistically significant correlation between the dose and change in CNTB summary score. From the 11 individual CNTB outcome parameters, only word list-delayed recall showed a statistically significant positive correlation with dose.

Correlations with inhibition of BuChE in plasma

The correlation between BuChE inhibition in plasma during 12 hours and inhibition of this enzyme in CSF was not statistically significant (Table 2). There was a trend, but no significant correlation, between BuChE inhibition in plasma and the change in CNTB summary score. Only one of 11 CNTB subtests (paired associate) showed a statistically significant association with BuChE inhibition in plasma (Table 3).

Correlations with inhibition of AChE in CSF

At baseline, i.e. before treatment with rivastigmine was initiated, the correlation between AChE and BuChE activities in CSF was 0.685 ($p < 0.01$), indicating a statistically significant, but not very tight association between the two enzymes before intervention with the ChE inhibitor. Following rivastigmine treatment, there was a similarly high correlation between inhibition of AChE and of BuChE in CSF. There was also a statistically significant

Table 3. Correlations between dose and enzyme inhibition and change in CNTB measures

	n[∞]	Dose	BuChE inhibition[†] Plasma	AChE inhibition[†] CSF	BuChE inhibition[†] CSF
CNTB – summary score	18	0.311	−0.401	−0.559*	−0.650**
	15	NA	NA	−0.567*	−0.683**
Boston naming test	18	−0.148	−0.038	−0.175	0.036
	15	NA	NA	−0.215	0.050
Choice reaction time – time[‡]	18	−0.175	0.086	0.403	0.501*
	15	NA	NA	0.476	0.650**
Finger tapping – both	18	−0.139	0.044	−0.108	−0.463
	15	NA	NA	−0.232	−0.483
Finger tapping – left	18	−0.143	0.182	−0.053	−0.350
	15	NA	NA	−0.179	−0.440
Finger tapping – right	18	−0.114	−0.063	−0.130	−0.470*
	15	NA	NA	−0.237	−0.440
Paired associate	18	0.222	−0.541*	−0.216	−0.526*
	15	NA	NA	−0.241	−0.420
Paired associate – delayed recall	18	0.044	−0.367	−0.360	−0.693**
	15	NA	NA	−0.406	−0.601*
Word list	18	0.330	0.092	−0.321	−0.333
	15	NA	NA	−0.405	−0.639*
Word list – delayed recall	18	0.533*	−0.049	−0.413	−0.032
	15	NA	NA	−0.508	−0.385
Simple reaction time	18	−0.090	0.044	0.400	0.460
	15	NA	NA	0.348	0.488
Visual memory	18	−0.092	0.093	−0.417	−0.524*
	15	NA	NA	−0.308	−0.509

[∞] Values presented are for n = 18 (three subjects with substituted CSF AChE and BuChE values at t_0) and for n = 15, excluding these three subjects (see text). [†] AUC values are negative for ChE inhibition as explained for Table 1. [‡] Reaction time scores decrease with improved performance, while other score increase. * $p < 0.05$; ** $p < 0.01$

correlation between AChE inhibition in CSF and the change in CNTB summary score (Table 3, Fig. 1). Several correlations between AChE inhibition in CSF and changes in subtests of the CNTB approached, but did not reach statistical significance.

Correlations with inhibition of BuChE in CSF

As indicated above, BuChE inhibition in CSF was not significantly correlated with the dose of rivastigmine nor the inhibition of BuChE in plasma (Table 2). There was, however, a statistically significant correlation with the inhibition of AChE in CSF. There was also a statistically significant correlation between BuChE inhibition in CSF and the change in CNTB summary score (Table 3, Fig. 2). Of the CNTB subtests, six were significantly correlated with inhibition of CSF BuChE: choice reaction time – time (for n = 18 and n = 15), finger

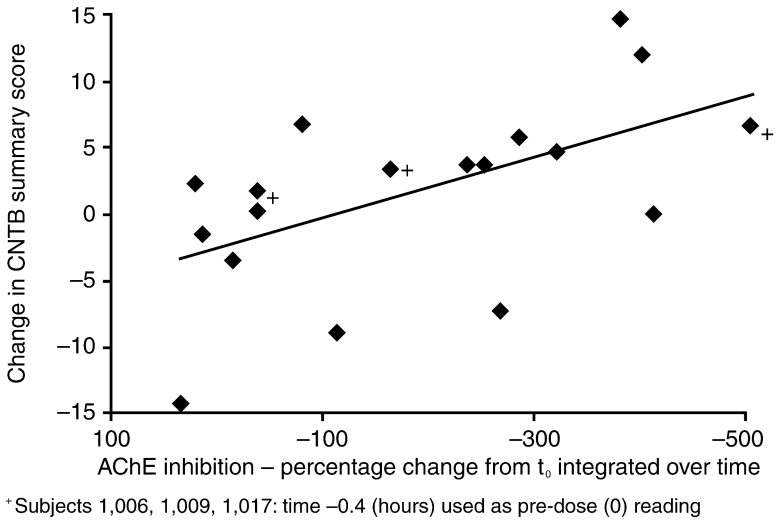

Fig. 1. Correlation between AChE Inhibition in CSF and change in CNTB summary score following rivastigmine treatment ($r = -0.56$, n = 18, $p < 0.05$)

tapping right (for n = 18), paired associate (for n = 18), paired associate-delayed recall (for n = 18 and n = 15), word list learning (for n = 15) and visual memory (for n = 18) (Fig. 3).

Another five correlations between BuChE inhibition in CSF and changes in CNTB subtest scores approached, but did not reach statistical significance.

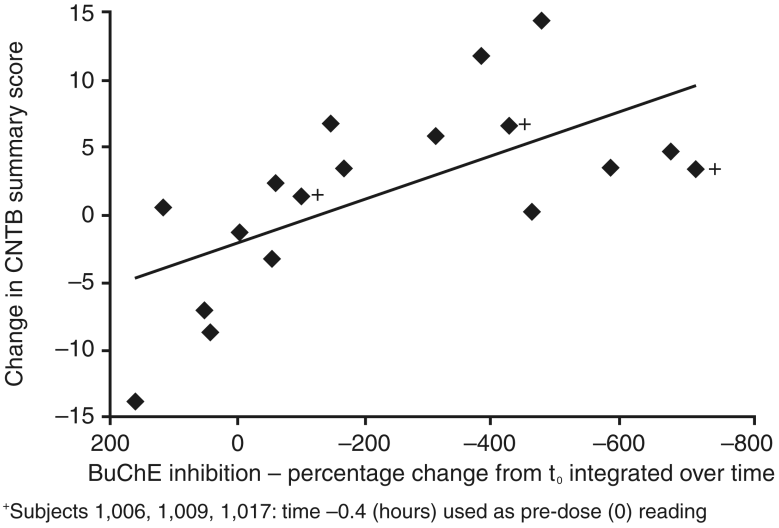

Fig. 2. Correlation between BuChE inhibition in CSF and change in CNTB summary score following rivastigmine treatment ($r = -0.65$, n = 18, $p < 0.01$)

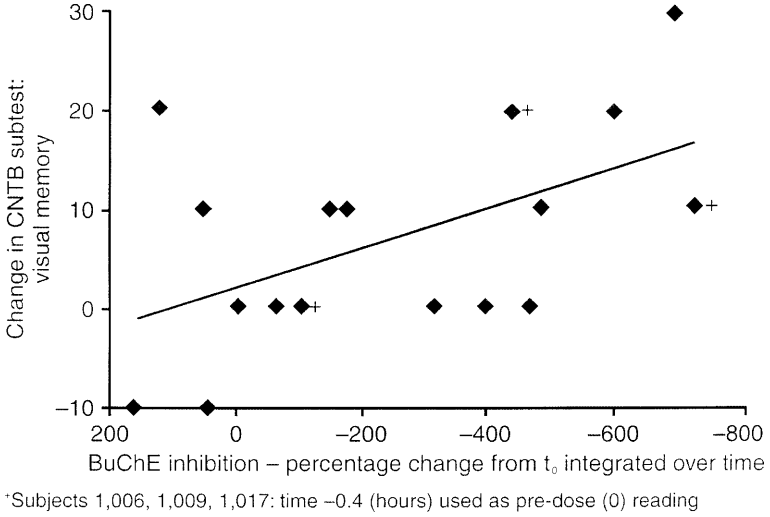

Fig. 3. Correlation between BuChE inhibition in CSF and change in CNTB subtest – Visual memory following rivastigmine treatment ($r = -0.52$, n = 18, $p < 0.05$)

Discussion

The study of Cutler et al. (1998) previously demonstrated that administration of rivastigmine 1–6 mg b.i.d. is associated with significant inhibition of AChE and BuChE in the CSF and plasma of patients with AD. The inhibition of BuChE was more pronounced in the CSF than in the plasma, suggesting a greater effect of rivastigmine in the central compartment compared with the periphery. This observation is consistent with the brain selectivity reported previously for this drug (Kennedy et al., 1999; Enz et al., 1993).

The current analysis confirms that the inhibition of plasma BuChE and CSF AChE are dose dependent over a 12 hour period following administration of rivastigmine. In contrast, the inhibition of BuChE in CSF does not show a clear dose dependency; this was mainly due to three patients who had very strong BuChE inhibition in CSF after 4 or 6 mg/day, which prevented a statistically significant correlation between the dose and inhibition of the enzyme. However, seven of the nine patients receiving daily doses of 8, 10 and 12 mg rivastigmine, demonstrated strong BuChE inhibition in the CSF.

AChE and BuChE in CSF at baseline, as well as the inhibition of the two enzymes by rivastigmine, were significantly inter-correlated, but the size of the correlations also indicates that the two enzymes are not tightly associated with one another. Both AChE and BuChE exist in multiple molecular forms, and the relative distribution of these is significantly changed in AD brain and CSF (Arendt et al., 1992). Rivastigmine has been shown, at least for AChE, to interact more potently with the G1 form, which is the form most predominant in AD brain (Enz et al., 1993).

Gobburu et al. (2001) did not find a significant association in the present patient group between the daily doses of rivastigmine and change in CNTB summary scores. This is mainly because several subjects treated with low

doses of rivastigmine showed some improvement on the CNTB summary score. Nevertheless, improvement on one subtest which measures learning/memory performance (word list learning – delayed recall) was significantly correlated with the dose.

The degree of AChE inhibition in CSF was significantly correlated with improvement of the CNTB summary score, although none of the 11 CNTB subtests showed a statistically significant association. Some of the non-significant trends suggest that AChE inhibition in CSF may be associated with increased speed/attention (simple and choice reaction time) and improved learning/memory performance (word list learning, delayed recall). The observation of a significant correlation between AChE CSF inhibition and the change in CNTB summary score is in contrast to findings published by Gobburu et al. (2001) upon analysis of the same data set. The different outcome is most likely due to the different statistical approach taken by Gobburu and colleagues: they modelled the time course for decline in CNTB scores taking into account disease progression and using data from a previous trial with tacrine to overcome the absence of placebo data. We believe that the present statistical approach, i.e. to consider correlations between changes from t_0 for enzyme activities and changes from baseline for cognitive performance, is more appropriate because this does not involve assumptions of CNTB decline over the relatively brief time course of the study.

The most striking finding of this study is the strong and consistent association between the inhibition of BuChE in CSF by rivastigmine and improved cognitive performance. This is evidenced by the significant correlation between BuChE inhibition in CSF and changes of the CNTB summary score, as well as the numerous significant correlations (and several non-significant trends) with CNTB subtests. Some of these tests measure speed/attention (choice reaction time and simple reaction time), others, motor speed (finger tapping) and still others, perhaps most importantly, functions of learning and memory (paired associate learning, paired associate learning with delayed recall, word list learning, visual memory).

In contrast, although BuChE in plasma was dose-dependently inhibited by rivastigmine, a significant correlation between CNTB summary score changes and peripheral BuChE inhibition was not observed. Of the 11 CNTB subtests, only one (paired associate learning with delayed recall) showed a significant association with BuChE inhibition in plasma. These results suggest that in AD patients, peripheral inhibition of BuChE is not a sensible biochemical correlate of changes of performance in speed/attention- and memory-related tasks.

Rivastigmine produced only small and transient reduction of CSF BuChE activity in healthy young volunteers (Kennedy et al., 1999), but strong and long-lasting inhibition of the enzyme in the CSF of AD patients (Cutler et al., 1998). In AD patients there was a low and statistically non-significant correlation between the inhibition of BuChE in plasma and in the CSF (Table 2). We also found that absolute levels of BuChE activity in plasma and CSF were not significantly associated at any time during the study (to be reported separately). This suggests that the BuChE activity and its changes measured in

CSF of AD patients does not have its origin from plasma, but most likely from the brain, presumably as a consequence of the higher BuChE activity found in the brain of AD patients (Perry et al., 1978; Arendt et al., 1992). The reduction by rivastigmine of BuChE activity in the CSF of AD patients and its correlates in cognitive performance would then be a reflection of the inhibition of BuChE occurring in the brain compartment rather than in the periphery.

Our data thus support a role for BuChE and its inhibition in the regulation of cognitive functions in AD. It may be hypothesized that the observations made in this study, and supported by data from animal studies in which BuChE-specific inhibitors provide cognitive benefit (Yu et al., 1999; Greig et al., 2001) imply that inhibition of BuChE in addition to AChE will be beneficial in the treatment of AD. The gradual decline in AChE activity and increase in BuChE activity during progression of AD may enable a non-selective inhibitor to augment and maintain central ACh levels along the continuum of disease.

A non-selective inhibitor such as rivastigmine might also provide greater disease modifying potential than inhibitors selective for AChE particularly at late stages of disease. Both AChE and BuChE activities are associated with the neurotoxic plaques characteristic of AD (Mesulam and Geula, 1994; Arendt et al., 1992; Guillozet et al., 1997). BuChE activity in particular appears to be involved in the transformation of plaques from a benign diffuse state to the compact malignant form (Guillozet et al., 1997). Non-selective inhibition may therefore help slow the formation of these plaques in the brains of patients with AD (Giacobini, 2000) thereby influencing disease progression.

A relationship between inhibition of CSF ChEs and cognitive function has also been suggested by preliminary data reported from a separate cohort of patients with AD. Treatment of patients with mild AD for 1 year with rivastigmine (3–12 mg/day) resulted in cognitive improvement at 3 and 6 months, and stabilisation of cognitive function at 12 months, which represent significant benefits compared with the decline in untreated patients (Nordberg et al., 2001a). In this study rivastigmine was associated with a greater mean dose-dependent inhibition of BuChE than AChE in the CSF (Nordberg et al., 2001b). Positron emission tomography (PET) brain imaging measurements made in the same patients indicated that the cognitive benefit attained after 1 year's treatment with rivastigmine coincided with improved glucose metabolism in cortical areas known to be involved in attention and working memory processes (Nordberg et al., 2001c). A region-specific effect in the cortex is consistent with pre-clinical studies which have indicated that rivastigmine exerts brain-region selectivity (Enz et al., 1993). The correlation between changes of the enzymes' activity and particular CNTB subtests in the present study also suggests effects of treatment on specific brain areas. The cognitive tasks with which CSF AChE and BuChE inhibition correlated best were tests of verbal and spatial memory, reaction time, and speed, which are functions associated with temporal, frontal and limbic regions of the brain. However no correlation was found on CNTB subtests of naming and visual matching, which are thought to probe parietal areas.

In summary, assessing the effects of rivastigmine treatment on the activity of both ChEs in CSF enabled preliminary observations to be made regarding the role of these enzymes in patients with AD. The correlation between CSF AChE and BuChE inhibition and improved cognitive function is supportive of a role for BuChE in addition to AChE in modulating the cholinergic systems important for maintaining cognitive function in AD. In addition these data highlight the value of assessing the effects of appropriate pharmacological interventions on both AChE and BuChE activity in CSF. This may enable determination of the most effective approaches to alleviating the symptoms of AD. Observations made in this study indicate that inhibition of both AChE and BuChE may be beneficial in treating the cognitive decline of AD.

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