Brain Cholinergic Function and Response to Rivastigmine in Patients With Chronic Sequels of Traumatic Brain Injury: A PET Study

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Objective: To investigate quantitative positron emission tomography (PET) findings and to study whether the cholinergic function differs between respondents to cholinergic medication versus nonrespondents. Setting: Outpatient clinic and university PET imaging center. Participants: We studied 17 subjects for more than 1 year after at least moderate traumatic brain injury. Ten of the subjects were respondents and 7 nonrespondents to cholinergic medication. Design: Cholinergic function was assessed with $[^{11}\text{C}]-\text{N}-\text{methylpiperidyl-4-acetate}$-[PET] ($[^{11}\text{C}]-\text{MP4A-PET}$), which reflects the activity of the acetylcholinesterase (AChE) enzyme. The subjects were PET scanned twice: without medication and after a 4-week treatment with rivastigmine 1.5 mg twice a day. Measures: Regional cerebral AChE activity was measured with PET. Results: At baseline Statistical Parametric Mapping analyses showed significantly lower AChE activity in respondents bilaterally in the frontal cortex as compared with nonrespondents. Region of interest (ROI) analysis revealed that the difference was most pronounced in the lateral frontal cortex ($-9.4\%, P = .034$) and anterior cingulate ($-6.0\%, P = .049$). After rivastigmine treatment, AChE activity was notably lower throughout the cortex in both respondents and nonrespondents, without significant differences between them. Conclusion: Our study suggests that frontal cholinergic dysfunction is associated with the clinical response to cholinergic stimulation in patients with traumatic brain injury. Key words: acetylcholine, acetylcholinesterase inhibitors, cognitive impairment, positron emission tomography, traumatic brain injury

Traumatic Brain Injury (TBI) is one of the most common causes of impaired function and disability, responsible for enormous human and economic costs.\(^1\) By the year 2020, it will surpass many diseases as the major cause of death and disability.\(^2\) Despite the economic and human burden of TBI, the mechanisms behind the chronic TBI sequels are poorly known. As the mechanisms are unknown, no drugs have been developed and registered to treat the cognitive and neuropsychiatric symptoms of TBI.

There is increasing evidence that the cholinergic system is involved in the cognitive sequels of TBI.\(^3\) The major cholinergic nuclei are situated in brain regions that are especially vulnerable in TBI because of the brain anatomy and injury biomechanics.\(^6\) Cholinergic neurons of the basal forebrain provide innervation to the neocortical mantle and hippocampus. These projections are important for attention, memory, and vigilance—functions commonly impaired after TBI.\(^8\) Reports that many patients with TBI and cognitive symptoms benefit from cholinergic medication support the hypothesis of cholinergic dysfunction in cognitive symptoms of TBI.\(^11\)–\(^14\)

There is increasing evidence of epidemiological and pathophysiological connections between Alzheimer disease (AD) and TBI. Cholinergic dysfunction seems to be a shared pathophysiology in both AD and TBI.\(^15\)
addition, β-amyloid accumulation, a hallmark of AD, is also seen in patients with TBI.\textsuperscript{16,17}

Positron emission tomography (PET) allows the in vivo quantification of acetylcholinesterase (AChE) activity with the tracer carbon-11-labeled N-methyl-4-piperidyl acetate (\textsuperscript{11}C-MP4A). MP4A is a lipophilic acetylcholine analogue with high AChE specificity. It serves as a substrate for AChE, and is hydrolyzed into a hydrophobic product that is trapped locally in the brain according to the distribution of enzyme activity. Earlier \textsuperscript{11}C-MP4A-PET studies of neurodegenerative processes such as AD, where the cholinergic system is affected, have revealed reduced AChE activities.\textsuperscript{18-20} It is also known that inhibitors of AChE, such as donepezil, rivastigmine, and physostigmine, decrease cortical AChE activity by 30\% to 50\% both in healthy subjects and in patients with AD.\textsuperscript{21,22} Induced inhibition of cortical AChE activity in subjects with AD has been associated with improvement in executive and attentional functions.\textsuperscript{23}

In our earlier \textsuperscript{11}C-MP4A-PET study, we found a widely lowered cortical AChE activity in subjects with cognitive sequelae of TBI compared with controls. That study demonstrated that a diffuse TBI with chronic cognitive consequences is likely to induce a wide cholinergic perturbation within the human cerebral cortex in vivo.\textsuperscript{24} The objective of the current study was to examine the brain cholinergic system using \textsuperscript{11}C-MP4A-PET by comparing 2 patient groups with chronic cognitive TBI sequelae with each other: those who had responded to central AChE inhibitors and those who had failed to respond.

**MATERIALS AND METHODS**

**Subjects**

The subjects with TBI were recruited from a database consisting of patients evaluated because of TBI at the Outpatient Clinic of the Department of Neurology, Turku University Central Hospital after the year 1993. At study entry, the database consisted of 1040 patients. The inclusion criteria were (1) Chronic sequelae of TBI with the presence of all 4 “core” symptoms typical for a chronic post-TBI syndrome: memory problems, fatigue, decreased initiation, and attentional deficits (emerged with the trauma), (2) more than 1 year postinjury and the above-mentioned symptoms subjectively unchanged for at least 3 months before the study entry, (3) mainly diffuse injury mechanism without large (>1 cm\(^3\)) focal traumatic brain lesions, based on brain computed tomography or magnetic resonance imaging (MRI), (4) an earlier treatment trial with rivastigmine with a minimum duration of 1 week and a minimum daily dose of 3 mg, and (5) at least 18 years of age. The following exclusion criteria were applied: (1) other diseases of the central nervous system or psychiatric disorders requiring medication, (2) current use of centrally acting drugs or drugs known to affect the cholinergic system that cannot be safely interrupted for at least 4 weeks, (3) contraindication for PET or MRI imaging, (4) uncertainty about the TBI diagnosis or about the etiology of the above-mentioned clinical symptoms, and (5) contraindication for rivastigmine treatment or adverse reactions during an earlier treatment trial with rivastigmine.

Altogether 38 subjects from the TBI database fulfilled the criteria of the study. These individuals were contacted and 19 of them agreed to participate and gave informed consent. Two persons discontinued the study. Thus, the final study group consisted of 17 subjects, 10 of them having shown subjective treatment response to central AChE inhibitors, and 7 of them having been nonrespondents. The respondents had been without cholinergic medications for at least 4 weeks before the study entry. Duration of rivastigmine treatment before the study varied from some weeks to several years. Nonetheless, it can be assumed that a 4-week washout period is long enough as there is no evidence of prolonged effects on AChE activity and rivastigmine has a short pharmacokinetic half-life.\textsuperscript{25} The drug response was systematically addressed in all subjects using a 5-step Likert scale from considerable harm to considerable benefit. All nonresponders reported neither harm nor benefit (mean 3.0 ± 0.0), whereas the responders all reported either clear or considerable benefit (mean 4.4 ± 0.52, with significant difference between groups \(P < .0002\)). The nonrespondents had experienced no notable effects from an at least 3 mg daily dose of rivastigmine, whereas the respondents had experienced a marked or very marked improvement in their daily functioning, with a typical treatment effect including diminished fatigue and improved attention. The treatment response had been confirmed in an interview with the patient and the proxy, and in case of any doubt about the cause of the subjective improvement, the treatment trial had been repeated with a washout period. This protocol had been routine in the evaluation of this off-label use of cholinergic medications in clinical practice. Moreover, the reappearance of the former effect was confirmed during this study, with all subjects experiencing a similar treatment effect to that found previously.

There were no significant differences between study participants and those who declined to participate in terms of severity of TBI (Glasgow Coma Scale [GCS] \(P = .664\), posttraumatic amnesia [PTA] \(P = .837\)), or outcome from TBI (Glasgow Outcome Scale, extended version [GOS-E], \(P = .965\) (\(\chi^2\) analysis), or age (\(P = .73\), \(t\) test). However, compared with the initial eligible patient population, the subjects of the study group were more often women than men (\(P = .015\)).
**Study design**

The patients were scanned twice, with and without medication. Four weeks before the time of the first PET ($^{11}$C-MP4A) scan, none of the participants was taking any medication known to affect cholinergic neurotransmission. For a minimum of 4 weeks before the second PET scan, the participants used rivastigmine medication at a dose of 1.5 mg twice a day. In further analyses the patients were separated into responding and nonresponding groups. To exclude structural lesions, for anatomical reference and volumetric analyses, each individual was scanned with MRI (Philips Gyroscan Intera 1.5 T) at the first PET scan.

**[11C]MP4A PET**

PET scanning protocol, blood sampling, and modeling of tracer kinetics are described in a previous study.24

**Standard protocol approvals, registrations, and patient consents**

Written informed consent was obtained from all patients participating in the study. The Conjoint Ethics Committee of Turku University and Turku University Central Hospital approved the study protocol.

**Statistical analyses**

Statistical analyses of region of interest (ROI) analyses were conducted with the SAS EG (V6.4, SAS Inc, Cary, North Carolina). A 1-way analysis of variance (ANOVA) was used for comparison of $k_3$ values of the first scan, without medication, between the patient groups. Group comparisons of nonrespondent and respondent groups, to compare baseline scan and scan with rivastigmine, were performed with ANOVA of repeated measurements. $P$ values less than .05 were interpreted as significant.

The statistical analyses of voxel-wise AChE activity were performed with Statistical Parametric Mapping (version SPM2; Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Prior to statistical analyses the parametric PET images were smoothed with a 14-mm FWHM Gaussian kernel. Group differences in the without-medication condition were analyzed with 1-way ANOVA and the effects of rivastigmine medication in respondent and nonrespondent groups with repeated-measures ANOVA (split-plot design). A binary mask including neocortical gray matter was applied for the SPM analyses. Cerebellum and striatum were excluded because in these regions the tracer uptake is too rapid for the assumptions of the kinetic model to be met. The ROI mask was generated with the WFU PickAtlas Tool (ANSIR Laboratory, Department of Radiologic Sciences, WFU School of Medicine, Winston-Salem, North Carolina). Group differences were tested with 1-tailed t-contrasts, and a cluster-level $P$ value corrected for multiple comparisons below .05 was regarded as significant.

**RESULTS**

**Demographics**

According to the duration of PTA, all subjects had at least moderate brain injury when using the criteria of the American Congress of Rehabilitation Medicine.26

<table>
<thead>
<tr>
<th>TABLE 1 Characteristics of the study subjects</th>
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</thead>
<tbody>
<tr>
<td><strong>Respondents</strong></td>
</tr>
<tr>
<td>$n = 10$</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Gender, male/female</td>
</tr>
<tr>
<td>GCS$^d$</td>
</tr>
<tr>
<td>PTA$^a$</td>
</tr>
<tr>
<td>1–7 d</td>
</tr>
<tr>
<td>1–4 wk</td>
</tr>
<tr>
<td>&gt;4 wk</td>
</tr>
<tr>
<td>Injury mechanism</td>
</tr>
<tr>
<td>Traffic</td>
</tr>
<tr>
<td>Fall</td>
</tr>
<tr>
<td>GOS-E</td>
</tr>
<tr>
<td>Years from TBI</td>
</tr>
</tbody>
</table>

Abbreviations: GCS, Glasgow Coma Scale; GOS-E, Glasgow Outcome Scale, extended version; PTA, posttraumatic amnesia; TBI, traumatic brain injury.

$^a$Duration of posttraumatic amnesia.

$^b$t-test.

$^c$Fisher exact test.

$^d$Glasgow Coma Scale at admission.
Using the GCS as a severity measure, the mean score was 10.3 (± 4.6 standard deviation, range 4–15). The 2 groups, nonrespondents and respondents, did not differ by gender ($P = .628, \chi^2$) or age ($P = .854, t$ test). The demographic and clinical characteristics of the study subjects are shown in Table 1.

### SPM analyses

SPM analyses showed a significant difference between the respondents and nonrespondents in the drug-naive condition bilaterally in the frontal cortex, the respondents having significantly lower AChE activity (see Figure 1). The analysis of posttreatment scans of TBI subgroups showed a significant lowering of the AChE activity widely in the neocortex after rivastigmine treatment compared with each group’s baseline. The analysis of posttreatment scans showed no significant difference in the level of $^{11}$C-MP4A binding between the respondents and nonrespondents.

### ROI analyses

ROI analyses included 6 main areas: (1) frontal cortex (including anterior cingulate and lateral frontal cortices), (2) posterior cingulate, (3) medial temporal cortex (including amygdala, hippocampus, and parahippocampal area), (4) lateral temporal cortex (including inferior, medial, and superior temporal gyri), (5) inferior part of parietal lobe (including angular and supramarginal gyri), and (6) occipital cortex.

In ROI analysis between the patient groups, a significantly lower AChE activity in the respondents as compared with nonrespondents was seen in the lateral frontal cortex ($−9.4 ± 9.2\%$, $P = .034$) and anterior cingulate ($−6.0 ± 4.7\%$, $P = .049$) in the drug-naive condition (see Table 2 and Figure 2). Comparison of the baseline scan to the scan with rivastigmine revealed that, in the respondents, rivastigmine induced the most marked reduction in AChE activity in the anterior cingulate ($−10.2 ± 4.6\%$, $P = .021$), lateral temporal ($−11.6 ± 6.4\%$, $P = .023$), and occipital ($−11.3 ± 21.8\%$, $P = .038$) cortices. In the nonrespondents, the decline in AChE activity was most marked in the lateral frontal ($−10.2 ± 10.4\%$, $P = .043$) and posterior cingulate ($−9.8 ± 8.4\%$, $P = .031$) cortices (see Figure 3). AChE activity was not statistically significantly different between the groups in the posttreatment scans. The inhibitory effect of rivastigmine on AChE activity did not differ between the respondents and nonrespondents in any brain region studied.

### DISCUSSION

Our study demonstrated that AChE activity was significantly lower bilaterally in the frontal cortex in those patients with at least moderate TBI who had benefitted from rivastigmine. So far there are no officially accepted medications for the cognitive impairment caused by TBI, but several mainly small-scale trials have suggested that central AChE inhibitors may be both efficient and safe in treating the cognitive sequelae of TBI. On the other hand, it appears that only some patients with TBI respond to these agents. These observations are compatible with our finding of significantly lower AChE activity in the lateral frontal and anterior cingulate cortices in the baseline scan in the respondents. As frontal dysfunction often lies behind the most disabling symptoms of TBI, the finding of a more profound frontal cholinergic dysfunction in the respondents fits nicely into this entirety. Whether the presence of relative frontal cholinergic hypofunction is a prerequisite for the therapeutic effect of central AChE inhibitors after TBI requires further studies. To clarify this, a prospective trial with rivastigmine for subjects with high or low frontal AChE activity would be needed. It is also interesting that the lowering effect of rivastigmine on AChE activity was similar in both respondents and nonrespondents. On the other hand, it seems that the AChE inhibition induced by rivastigmine was smaller in the respondents...
Figure 2. AC and LFC activities in ROI analyze in no-medication state. AC, anterior cingulate; LFC, lateral frontal cortex.

Figure 3. AC and LFC activities in ROI analyze in with-medication state. AC, anterior cingulate; LFC, lateral frontal cortex.
Results of the ROI analyses in subjects with TBI, both with and without medication compared between respondents and nonrespondents to cholinergic stimulation

<table>
<thead>
<tr>
<th>Region</th>
<th>No medication</th>
<th>With medication</th>
<th>Respondents relative to nonrespondents</th>
<th>Nonrespondents relative to nonrespondents</th>
<th>Respondents relative to nonrespondents</th>
<th>Nonrespondents relative to nonrespondents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Mean (±SD)</td>
<td>P&lt;sub&gt;a&lt;/sub&gt;</td>
<td>P&lt;sub&gt;b&lt;/sub&gt;</td>
<td>P&lt;sub&gt;c&lt;/sub&gt;</td>
<td>P&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>3.8% (24.9)</td>
<td>6.67 ± 10.2</td>
<td>0.11</td>
<td>0.036</td>
<td>0.034</td>
<td>0.032</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>3.8% (24.9)</td>
<td>6.67 ± 10.2</td>
<td>0.11</td>
<td>0.036</td>
<td>0.034</td>
<td>0.032</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>6.9% (10.4)</td>
<td>12.7 ± 10.2</td>
<td>0.032</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Inferior part of parietal lobe</td>
<td>6.9% (7.4)</td>
<td>5.89 ± 10.2</td>
<td>0.033</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>4.8% (9.0)</td>
<td>6.29 ± 10.2</td>
<td>0.046</td>
<td>0.012</td>
<td>0.017</td>
<td>0.017</td>
</tr>
</tbody>
</table>

**Table 2.** Results of the ROI analyses in subjects with TBI, both with and without medication compared between respondents and nonrespondents to cholinergic stimulation.

Abbreviation: SD, standard deviation.

*a (scan 2 / scan 1) × 100.

*b One-way ANOVA.

*c Repeated measurement ANOVA.

Because the difference between the patient groups disappeared in the posttreatment scans. However, after treatment there was still a trend for smaller values especially in the lateral frontal cortex in the respondents versus nonrespondents, although this difference did not reach statistical significance.

The dose of rivastigmine used in our study was fairly low. This probably explains why the degree of AChE inhibition (around 10%) was lower compared with that found in studies in AD (around 30%), where clearly higher doses were applied. We chose to use low doses of rivastigmine based on the large clinical experience (>500 patients at the time of the study) in the use of cholinergic agents in patients with TBI. There is a clear difference in the clinical response to cholinergic stimulation in patients with past TBI and AD: the patients with TBI seek for the response immediately and with low doses, usually without further benefit from higher doses but increased risk of adverse effects.

Our study has some limitations, the most important of which is its small sample size, the usual problem in PET studies. Second, the patients were selected from an outpatient clinic material and therefore the mildest and most severe injuries may be underrepresented, which explains why the results cannot be generalized to all patients with past TBI. Moreover, although there were no statistically significant differences in the demographic properties between the responders and nonresponders, there were almost significant differences in their outcomes as well as in the time from injury. We cannot fully exclude the possibility that these differences could in part contribute to the current results. However, we think this is unlikely because based on our earlier studies as well as the wide clinical experience with rivastigmine in TBI, neither of them has been connected with the response. Most respondents used rivastigmine at the time of recruitment but discontinued the medication for 4 weeks in the study. There is no evidence that rivastigmine could have long-lasting effects on AChE activity, but this possibility should not be fully excluded.

Furthermore, we did not study the dose-response with rivastigmine and the assessment of the drug response lacked blinding, as well as formal cognitive testing. However, the previous clinical experiences of treatment response were replicated in this trial and formal cognitive assessments have usually been insensitive to show significant changes despite marked subjective benefit, mainly because of the great interindividual and intraindividual variability in this patient group. Lack of systematic evaluation of efficacy using cognitive testing may also be considered a major shortcoming, but our earlier studies as well as others have shown that cognitive testing is often insensitive due to several confounders such as daily...
variability, mood, tiredness, and motivation. In addition, for symptomatic medications a subjective benefit is the only determinant of clinical usability.

In all drug trials, a placebo effect must also be taken into account. However, before leaving rivastigmine in off-label use, we test the clinical efficacy repeatedly. Moreover, most patients had also participated in an earlier double-blinded trial, and the reappearance of the former effect was confirmed during this study. Therefore, it seems unlikely that a placebo effect could explain these benefits. It is also possible that some of the beneficial effects of rivastigmine are not directly mediated but emerge as a secondary response to changes in the cholinergic system.

AChE inhibitors are shown to be an effective symptomatic treatment in AD, and there are also several lines of evidence showing that some patients with TBI benefit from these agents. Most studies of AChE inhibitors in TBI have been open-labeled and small. Therefore, there is still a clear need for a large double-blinded study of AChE inhibitors to determine the chronic sequel of TBI. However, our study suggests that only some patients with past TBI respond, which is why selection of study subjects may be a challenge. The absence of therapeutic effect may be attributable to the highly heterogeneous pathophysiological nature of TBI. This highlights the need for reliable methods of patient stratification for future TBI trials.

REFERENCES