Cholinergic dysfunction after traumatic brain injury

Preliminary findings from a PET study

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ABSTRACT

Objective: There is evidence that the cholinergic system is frequently involved in the cognitive consequences of traumatic brain injury (TBI). We studied whether the brain cholinergic function is altered after TBI in vivo using PET.

Methods: Cholinergic function was assessed with [methyl-^{11}C]*N*-methylpiperidyl-4-acetate, which reflects the acetylcholinesterase (AChE) activity, in 17 subjects more than 1 year after a TBI and in 12 healthy controls. All subjects had been without any centrally acting drugs for at least 4 weeks.

Results: The AChE activity was significantly lower in subjects with TBI compared to controls in several areas of the neocortex (−5.9% to −10.8%, $p = 0.053$ to 0.004).

Conclusions: Patients with chronic cognitive symptoms after TBI show widely lowered AChE activity across the neocortex. *Neurology*® 2011;76:1046–1050

GLOSSARY

AChE = acetylcholinesterase; **MNI** = Montreal Neurological Institute; **ROI** = region of interest; **SPM** = statistical parametric map; **TAC** = time-activity curve; **TBI** = traumatic brain injury.

The mechanisms of chronic traumatic brain injury (TBI) consequences are poorly understood, but the cholinergic system may play an important role in TBI-related cognitive deficits.[1-3] The major cholinergic centers are situated in regions that are especially vulnerable in TBI because of the brain anatomy and injury biomechanics.[4,5] Vigilance, attention, and memory are at least partly cholinergically mediated and are frequently compromised after TBI.[6-8] Both experimental TBI models and postmortem human studies of patients with TBI have shown involvement of the cholinergic system.[9-13] Cholinergic stimulation may be beneficial in chronic cognitive TBI symptoms,[14-19] although the results from randomized controlled trials have remained modest.[20,21]

In vivo evaluation of acetylcholinesterase (AChE) activity with PET is based on the use of N-methylpiperidyl ester that serves as a substrate for AChE. The ester is hydrolyzed to a hydrophilic product that is trapped locally in the brain according to the distribution of enzyme activity. This method has been validated in animals and extended to human studies.[22-24] AChE inhibitors, such as donepezil and rivastigmine, decrease cortical AChE activity by 30%–50% in healthy subjects or in patients with AD.[23,25]

The aim of this study was to test the hypothesis that patients with chronic cognitive symptoms after TBI show cholinergic hypoactivity, by examining the in vivo cholinergic system with PET, compared to healthy controls.

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 1993. At study entry, the database consisted of 1,040 patients. The inclusion criteria were 1) chronic consequences of TBI with the presence of all 4 core symptoms typical for a postconcussive syndrome, namely memory problems, fatigue, decreased initiation, and attention deficits (emerged with

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the trauma); 2) stable symptoms for at least 3 months, more than 1 year postinjury; 3) mainly diffuse injury mechanism without large (>1 cm^3) local contusions or brain tissue destruction, based on brain CT or MRI; and 4) at least 18 years of age. The following exclusion criteria were applied: 1) other diseases of the CNS or psychiatric disorders requiring medication; 2) current use of centrally acting drugs that cannot be safely interrupted for at least 4 weeks; 3) contraindication for PET or MRI; or 4) uncertainty about the TBI diagnosis or the etiology of the symptoms. The diagnosis of TBI was based on a thorough clinical workup including 1) fulfillment of American Congress of Rehabilitation Medicine diagnostic criteria for TBI[26]; 2) careful evaluation (using also other than the patient's subjective report) of preinjury health and function; 3) extensive neuropsychological examination; 4) structural brain imaging with MRI; 5) careful evaluation of the injury mechanism and the clinical stage and symptoms during the acute phase; and 6) exclusion of other likely explanations for the patient's symptoms. All these were done by a very experienced neurologist in TBI evaluation. Cases with any diagnostic uncertainty were excluded.

The control group was recruited from healthy volunteers matched for age and sex. The exclusion criteria were 1) contraindication for PET or MRI; 2) current use of centrally acting drugs; 3) previous head injuries resulting in any loss of consciousness, any duration of amnesia, or other postconcussive symptoms lasting more than 1 week; or 4) signs of brain pathology in MRI.

Altogether 38 subjects from the TBI database fulfilled the criteria of the study. These were contacted and 19 of them agreed to participate and gave informed consent. Two persons discontinued the study because of subjective inconvenience during PET imaging. Thus, the final study group consisted of 17 subjects with TBI. The study subjects did not differ from those who were eligible in terms of age, TBI severity, or TBI outcome, but there were more females in the final study group ($p = 0.015$). Twelve healthy volunteers were studied as controls. The demographic and clinical characteristics of the study subjects are shown in table 1.

Table 1 Characteristics of the study subjects

	Subjects with TBI (n = 17)	Controls (n = 12)	*p* Value[a]
Age, y, mean ± SD	44.8 ± 12.2	48.4 ± 13.9	0.449[b]
M/F	6/11	5/7	0.858[c]
GCS, mean ± SD[d]	10.3 ± 4.6		
Duration of PTA, n (%)			
1–7 d	4 (23)		
1–4 wk	9 (53)		
>4 wk	4 (23)		
Injury mechanism, n (%)			
Traffic	14 (82)		
Fall	3 (18)		
Years from TBI, mean ± SD	9.9 ± 6.7		
GOS-E, mean ± SD	5.6 ± 1.0		

Abbreviations: GOS-E = Glasgow Outcome Scale extended version; PTA = posttraumatic amnesia; TBI = traumatic brain injury.
[a] Subjects with TBI vs controls.
[b] *t* Test.
[c] χ^2.
[d] Glasgow Coma Score at admission.

Study design. A [methyl-^{11}C]*N*-methylpiperidyl-4-acetate (^{11}C-MP4A, a lipophilic acetylcholine analog with high AChE specificity) PET imaging scan was performed in all participants, including controls. All subjects were scanned after at least 4 weeks without any centrally acting drugs. The analyses were done by comparing all subjects with TBI to controls.

Scanning protocol and blood sampling. The radiochemical synthesis and quality control of ^{11}C-MP4A have been described in detail elsewhere.[25] The PET imaging was performed with a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, WI). Cannulas were placed in an antecubital vein for ^{11}C-MP4A injection. At the start of the scan, ^{11}C-MP4A was injected IV as a constant bolus during 80 s, and the radioactivity was measured in a consecutive series of 22 frames (1×30 s, 4×15 s, 5×30 s, 2×60 s, 2×120 s, 6×300 s, 2×600 s) with a total scan duration of 60 minutes.

All subjects were scanned with a Philips Gyroscan Intera 1.5 T CV Nova Dual scanner (Philips, Best, the Netherlands). Axial T2 and coronal fluid-attenuated inversion recovery sequences were obtained to visualize TBI lesion load and, in controls, to exclude any brain pathology. A whole-brain T1-weighted 3-dimensional dataset was acquired in the transverse plane.

Image preprocessing. PET and MRI were processed with in-house software[27] and Statistical Parametric Mapping version 2 (SPM2) (Wellcome Department of Cognitive Neurology, London, UK[28]) running on Matlab 6.5 (The MathWorks, Natick, MA). First, the images were movement corrected as described previously[29]; frame 14 (from 6 to 8 minutes from injection) was used as the reference to which other frames were individually realigned, except for the first 10 frames (0 to 4 minutes from injection), where a summated image over the frames was used to calculate the transformation and the obtained parameters were applied to each frame separately.

For the spatial normalization of PET images, a ligand-specific template for ^{11}C-MP4A was generated using MRI-aided procedures as described in detail earlier.[30,31] In brief, first the individual MRIs were coregistered to summated ^{11}C-MP4A images and then the MRIs were normalized to a T1-weighted MRI template in the Montreal Neurological Institute (MNI) space. Using the acquired transformation parameters, ^{11}C-MP4A summated images were normalized and the mean of these normalized images was averaged with its mirror copy (left-right flipped image). This symmetric mean ^{11}C-MP4A summated image was smoothed using an 8-mm Gaussian kernel, to create a ligand-specific template. Individual dynamic PET images were spatially normalized to the ligand-specific template with parameters estimated from summated ^{11}C-MP4A images in native space.

MRI coregistered to the summated PET image in native space were also written in the MNI space with the parameters estimated from the individual's summated PET image to the ligand-specific template. A set of regions of interest (ROIs) was defined from the average of these normalized MRI using Imadeus software (version 1.50, Forima Inc., Turku, Finland), and the location of ROIs was visually checked to match each normalized PET image. ROIs were delineated bilaterally on the frontal cortex, lateral temporal cortex, medial temporal lobe, inferior part of the parietal lobe, occipital cortex, posterior cingulatum, and putamen. The ROIs were then transferred to the corresponding planes in the normalized dynamic PET images and time-activity curves (TAC) for ROIs were calculated.

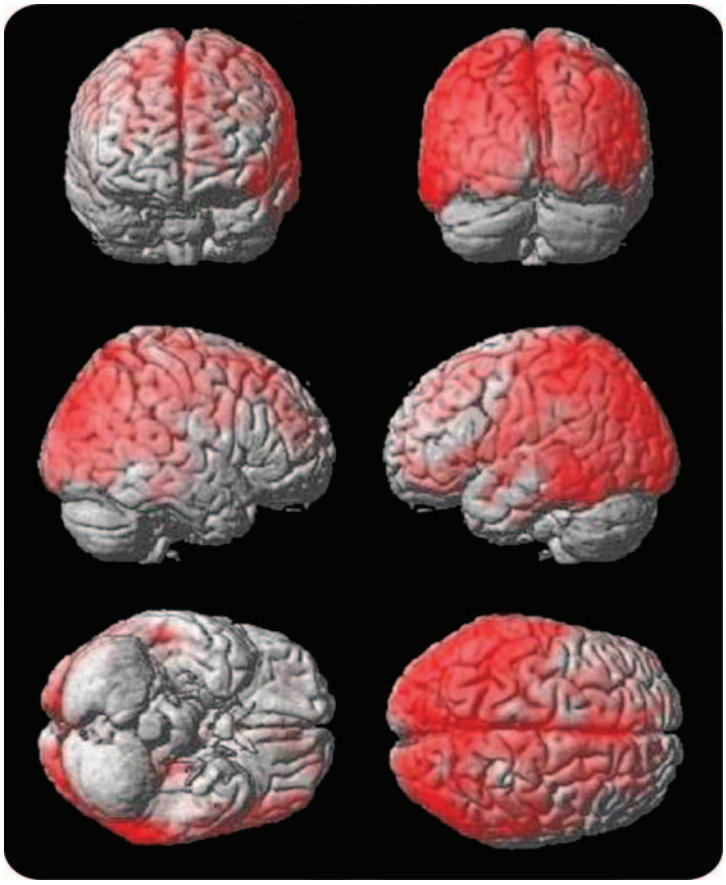
Modeling of tracer kinetics. AChE activity was calculated applying the transport-limited reference tissue model for irre-

versible uptake, in which the k_3 rate constant represents the hydrolysis rate of the tracer in tissue, and thus the local AChE activity. This model is based on the reference tissue compartment model, but here it is assumed that in the reference tissue tracer the metabolism is fast, and thus the tracer uptake is limited only by transport into tissue.[32,33] The putamen was used as reference tissue. The model was solved for ROI-TACs to calculate regional AChE activity, and for each image pixel separately to produce parametric images representing AChE activity.

Standard protocol approvals, registrations, and patient consents. Written informed consent was obtained from all patients participating in the study. The study protocol was approved by the Conjoint Ethics Committee of Turku University and Turku University Central Hospital.

Statistical analyses. Statistical analyses of ROI analyses were conducted with the SAS System for Windows (V9.1, SAS Inc., Cary, NC). To test for the differences in demographic variables, χ^2 tests were applied for categorical variables and *t* test for continuous variables. One-way analysis of variance was used for the comparison of regional AChE activity between the subjects with TBI as a whole group and control subjects. *p* Values less than 0.05 were interpreted as significant.

Figure **Statistical parametric map (SPM) of t contrast between subjects with traumatic brain injury (TBI) and controls**



Subjects with TBI had significantly lower acetylcholinesterase (AChE) activity compared to controls in several neocortical areas. Height threshold T = 1.8 and extent threshold k = 2,000 correspond to cluster-level family-wise error corrected $p < 0.01$. The red scale indicates the level of statistical significance of the difference in AChE activity (solid red indicates the most significant difference).

The statistical analyses of voxel-wise AChE activity were performed with SPM2. Prior to statistical analyses, the parametric PET images were smoothed with a 14-mm full width at half maximum Gaussian kernel. A binary mask including neocortical gray matter was applied for the SPM analyses. The 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 a WFU PickAtlas Tool.[34] Group differences were tested with *t* contrasts, and cluster-level *p* values corrected for multiple comparisons below 0.01 were regarded as significant. The SPM results were localized with MSU Space Utility.[35]

RESULTS The subjects with TBI and controls did not differ from each other in regard to gender and age (table 1). According to the duration of posttraumatic amnesia, all subjects with TBI had at least moderate injury when using the criteria of the American Congress of Rehabilitation Medicine.[35] Using Glasgow Coma Score as a severity measure, the mean score was 10.3 (±4.6 SD, range 4–15).

SPM and ROI analyses. The SPM analysis showed that the AChE activity was significantly lower in subjects with TBI than in controls in several areas of the neocortex (figure), most pronouncedly in the parieto-occipital regions. In ROI analysis, the AChE activity was lower in subjects with TBI compared to controls in all ROIs, except the medial temporal cortex (table 2). If corrected for multiple comparisons, the results of the parietal and cingulate cortices remained significant (table 2).

DISCUSSION Lower cortical AChE activity in subjects with TBI compared to controls agrees with evidence from earlier experimental and postmortem studies that have demonstrated cholinergic dysfunction after TBI,[9-13] as well as with a recent observation of widely increased cortical glucose metabolism in patients with TBI after treatment with the central AChE inhibitor donepezil.[36] This suggests that diffuse TBI with chronic cognitive consequences is likely to induce a wide cholinergic perturbation within the human cortex in vivo.

Although there are no officially accepted medications for the cognitive impairments caused by TBI, several clinical trials suggest that central AChE inhibitors may be both effective and safe in treating the cognitive consequences of TBI.[15-19,37] Conversely, it appears that only a portion of subjects with TBI responds to these agents.[20,21] It is unclear whether the nature and mechanism of the TBI influences this response.

The reason for the lowered AChE activity in the subjects with TBI with chronic consequences remains speculative. While altered AChE activity can be taken as a marker of general cholinergic dysfunc-

Table 2 Results of the ROI analyses in subjects with TBI compared to control subjects

Region	Subjects with TBI relative to controls, % (±SD)	*p* Value[a]	Corrected *p* value[b]
Frontal cortex			
Anterior cingulatum	−5.9 (6.1)	0.048	0.336
Lateral frontal cortex	−7.1 (7.9)	0.035	0.245
Temporal cortex			
Medial temporal cortex	−7.6 (3.8)	0.053	0.371
Lateral temporal cortex	−8.2 (7.6)	0.017	0.119
Parietal cortex, inferior part	−9.9 (10.0)	0.005	0.035
Occipital cortex	−10.5 (16.8)	0.009	0.063
Posterior cingulatum	−10.8 (8.7)	0.004	0.028

Abbreviations: ROI = region of interest; TBI = traumatic brain injury.
[a] *t* Test.
[b] With Bonferroni correction.

tion, the mechanism of this dysfunction cannot be inferred directly from ^{11}C-MP4A PET studies. Those brain areas that contain the majority of ascending cholinergic projection neurons are especially vulnerable in TBI.[5] Therefore, the lowered AChE activity may stem from the dysfunction of these neurons, either in the form of neuronal loss or functional deficit. Conversely, especially diffuse injury mechanisms affect large cortical areas, with the most profound pathology at the junction of gray and white matter.[38] As AChE is localized both presynaptically and postsynaptically in the cholinergic synapses, lowered activity may stem from an injury either to the presynaptic ascending neurons or postsynaptic cortical neurons or both.[39] Also modern MRI methodologies have shown the involvement of both white matter tracts and cortical structures in TBI pathology, with the frontal regions being especially vulnerable even in milder cases.[40] The relationships between neuronal damage and the cholinergic dysfunction could be studied in vivo by analyzing ^{11}C-MP4A PET images in conjunction with structural MRI and diffusion tensor imaging, which is currently being done within our group.

There are limitations to our study that should be considered. First, the sample sizes of the controls and the subjects with TBI were relatively small due to the strict inclusion and exclusion criteria. Second, as a result, these subjects represent only a certain type of TBI, so the results cannot be generalized to all patients with TBI. While small sample size is a limitation, in studies using radioactive isotopes, the number of subjects and the number of scans should be kept as low as possible to obtain sufficient information to address the hypotheses, but avoiding unnecessary exposure to ionizing radiation. Although this sample size proved to be sufficient to reveal differences between subjects and controls in several brain areas (thus supporting the hypothesis of our study), failure to find anticipated differences in cortical AChE activity in some brain areas (e.g., medial temporal lobe) most likely reflects inadequate power to do so.

Beneficial effects of the central AChE inhibitors have been widely studied in AD and now there is also slowly accumulating evidence that these agents may also relieve cognitive impairments after TBI. It would be interesting to study how large a percentage of patients with chronic TBI consequences have cholinergic dysfunction, and how its presence might correlate with the symptom profile and outcome. In further studies, we hope to clarify whether the difference between the central AChE inhibitor respondents and nonrespondents after TBI can be assessed with neuropsychological measures or modern MRI methods, correlating the results also to the ^{11}C-MP4A PET images in the same subjects.

DISCLOSURE

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