Association of Increased Cortical Soluble \( \text{A}\beta_{42} \) Levels With Diffuse Plaques After Severe Brain Injury in Humans

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**Background:** Traumatic brain injury (TBI) is an environmental risk factor for developing Alzheimer disease. This may be due, in part, to changes associated with \( \beta \)-amyloid (A\( \beta \)) plaque formation, which can occur within hours after injury, regardless of the patient’s age. In addition to being precursors of toxic fibrils that deposit into plaques, soluble (nonfibrillar) A\( \beta \) peptides are posited to disrupt synaptic function and are associated with cognitive decline in Alzheimer disease. Changes in soluble A\( \beta \) levels and their relationship to A\( \beta \) plaque formation following TBI are unknown.

**Objective:** To quantify brain tissue levels of soluble A\( \beta \) peptides and their precursor protein in relation to A\( \beta \) plaque formation after TBI in humans.

**Design:** Surgically resected temporal cortex tissue from patients with severe TBI was processed for biochemical assays of soluble A\( \beta \) peptides with COOH-termini ending in amino acid 40 (A\( \beta_{40} \)) or 42 (A\( \beta_{42} \)) and A\( \beta \) precursor protein to compare patients with cortical A\( \beta \) plaques and those without.

**Patients:** Nineteen subjects admitted to the University of Pittsburgh Medical Center for treatment of severe closed head injury.

**Results:** Patients with severe TBI and cortical plaques had higher levels of soluble A\( \beta_{42} \) but not A\( \beta_{40} \); half of them were apolipoprotein E (APOE) \( \varepsilon4 \) allele carriers. The lowest A\( \beta \) levels were in 1 patient without plaques who was the only subject with an APOE \( \varepsilon2 \) allele. \( \beta \)-Amyloid precursor protein levels were comparable in the 2 TBI groups.

**Conclusions:** Selective increases in soluble A\( \beta_{42} \) after TBI may predispose individuals with a brain injury to Alzheimer disease pathology. This may be influenced by the APOE genotype, and it may confer increased risk for developing Alzheimer disease later in life.

Arch Neurol. 2007;64:541-544

The molecular mechanism of A\( \beta \) plaque formation after TBI in humans is not known, but likely involves altered A\( \beta \) precursor protein (APP) production and/or metabolism, which occurs in animal TBI models. After TBI in humans, accumulation of APP fragments (including A\( \beta \)) in damaged axons and plaques has been observed using immunohistochemical techniques. Reduced levels of soluble A\( \beta \) have been detected in cerebrospinal fluid, suggesting that reduced A\( \beta \) clearance may contribute to increased A\( \beta \) levels in brain tissue. To date, there have been no quantitative biochemical assessments of A\( \beta \) peptides and APP in human brain tissue after TBI. Given the potential synaptotoxic effects of soluble A\( \beta \) peptides and plaque-associated fibrils in AD, understanding postinjury changes in soluble A\( \beta \) could provide insight into the pathologic sequelae of
TBI heretofore undetected in immunohistochemical studies. Accordingly, we examined changes in soluble Aβ peptides and APP levels relative to Aβ plaque deposition following severe TBI in humans.

### METHODS

This study included 19 patients with TBI (Table) admitted to the University of Pittsburgh Medical Center for treatment of severe closed head injury (Glasgow Coma Scale score <9). Studies were approved by the institutional review board at the University of Pittsburgh; written informed consent was obtained from family members. The details of patient management were published previously. All patients underwent decompressive craniectomy to relieve intractable cerebral swelling. Temporal cortex tissue removed for this purpose (that would normally be discarded) was used. One portion of each sample was fresh frozen and stored at −80°C for biochemistry; the adjacent tissue was fixed with 4% paraformaldehyde and processed for Aβ immunohistochemistry in our previous study, which identified 2 groups of patients with TBI: those with diffuse Aβ plaques in the temporal cortex (plaque positive) and those lacking Aβ plaques (plaque negative) (Table). Another set of frozen temporal cortex tissue samples was obtained through the University of Pittsburgh Alzheimer Disease Research Center Brain Bank post mortem from 18 clinically diagnosed and neuropathologically confirmed patients with AD (mean±SD, age, 78.9±8.7 years; postmortem interval, 7.3±3.3 hours), and 5 neurologically healthy elderly individuals (mean±SD, age, 74.8±5.9 years; postmortem interval, 7.8±3.7 hours). Because control surgical samples are not available from healthy young people, these older autopsy subjects were used to validate our biochemical measurements. Soluble Aβ peptides were assayed using fluorescent-based enzyme-linked immunosorbent assay (Biosource, Camarillo, Calif) with a capture antibody specific for the NH2-terminus of Aβ (amino acids 1-16) and detection antibodies specific for Aβ peptides Aβ1-40 or Aβ1-42, β-Amyloid enzyme-linked immunosorbent assay levels were expressed as picomoles of Aβ per gram of the wet weight of tissue. The APP was assayed by Western blot using an NH2-terminus–specific monoclonal APP antibody with a 1:1000 dilution (22C11; Roche, Basel, Switzerland), as described previously. Statistical analyses were performed using the Mann-Whitney test and Spearman rank correlation test. Two-tailed P values less than .05 were considered statistically significant.

### RESULTS

Demographic data were similar between the 2 TBI groups (Table), with the exception of having fewer women than men and a lower frequency of the apolipoprotein E (APOE) ε4 allele in the Aβ plaque–negative group. Fifty percent of the patients with severe TBI and cortical plaques carried the APOE ε4 allele, a rate that was higher than expected, because approximately 20% to 25% of the white population is APOE ε4 positive. In the plaque-negative group, APOE ε4 allele frequency was lower (11%). Soluble Aβ1-42 and Aβ1-40 were detected in the temporal cortex of all patients with TBI, and there was a significant direct correlation between levels of these 2 Aβ species (Spearman rank correlation r = 0.68; P = .0005). Soluble Aβ1-42 levels were significantly higher in the plaque-positive group compared with the plaque-negative group (P = .0098) (Table), while Aβ1-40 levels were comparable, resulting in a greater Aβ1-42/Aβ1-40 ratio in the plaque-positive group (P = .012). There was a trend toward a correlation of higher soluble Aβ levels with greater Aβ plaque load in plaque-positive cases (not shown). The lowest levels of soluble Aβ1-42 and Aβ1-40 (10-fold and 5-fold lower, respectively, than the means of the population) were in the only APOE ε2 patient (APOE 2/3 plaque negative). As validation of the Aβ enzyme-linked immunosorbent assay procedure, Aβ1-43 and Aβ1-40 levels in the temporal cortex of patients with AD (mean±SD, Aβ1-42, 20.17±4.08 pmol/g of wet weight of tissue; Aβ1-40, 6.47±1.48 pmol/g of wet weight of tissue) and older control subjects (mean±SD, Aβ1-42, 0.86±0.13 pmol/g of wet weight of tissue; Aβ1-40, 0.33±0.05 pmol/g of wet weight of tissue) were similar to those reported previously. To examine the effect of time on changes in soluble Aβ levels following TBI, we correlated Aβ levels with patients’ time intervals between injury and surgical tissue extraction. Longer intervals between injury and extraction were directly correlated with higher Aβ1-42 (r = 0.50; P = .029) but not Aβ1-40 levels. Western blot analysis of APP revealed 2 major bands, at 115 and 105 kd, corresponding to expected molecular weights of major full-length APP isoforms (not shown). Both bands were prominent in all patients with TBI and

### Table. Patient Demographics and Results of Aβ1-40 and Aβ1-42 ELISA Analyses in Temporal Cortex Tissue Surgically Extracted From Patients With Severe TBI, With and Without Aβ-Immunoreactive Plaques

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aβ Plaque Positive (n=6)</th>
<th>Aβ Plaque Negative (n=13)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>46.2 (12)</td>
<td>42.9 (17.8)</td>
<td>.97</td>
</tr>
<tr>
<td>Female sex, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCS score</td>
<td>50</td>
<td>23</td>
<td>NA</td>
</tr>
<tr>
<td>Interval between injury and tissue extraction, h</td>
<td>6.2 (2.6)</td>
<td>4.7 (1.7)</td>
<td>.22</td>
</tr>
<tr>
<td>APOE ε4 carrier, %</td>
<td>50</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>Aβ1-42 level, pmol/g of wet tissue</td>
<td>2.91 (1.21)</td>
<td>0.54 (0.09)</td>
<td>.009</td>
</tr>
<tr>
<td>Aβ1-40 level, pmol/g of wet tissue</td>
<td>0.54 (0.12)</td>
<td>0.30 (0.05)</td>
<td>.01</td>
</tr>
<tr>
<td>Aβ1-42/Aβ1-40 ratio</td>
<td>4.65 (1.12)</td>
<td>1.86 (0.27)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Aβ, β-amyloid; APOE ε4, apolipoprotein E ε4; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Scale; NA, not applicable; TBI, traumatic brain injury.

*Values are mean (SD), unless otherwise indicated.
†All patients who were not carriers of APOE ε4 had an APOE 3/3 genotype, except for 1 case (APOE 2/3 genotype) in the plaque-negative group.
did not differ significantly between plaque-positive (mean ± SD, 5410 ± 3653 optical density units) and plaque-negative TBI groups (mean ± SD, 4351 ± 1009 optical density units).

**COMMENT**

The selective increase in Aβ1-42 levels in patients with severe TBI and cortical plaques could explain the predominance of Aβ1-42 over Aβ1-40 plaques reported in human TBI autopsy series and in our biopsy immunohistochemical study. Aβ1-42 is more prone to aggregation and may initiate plaque formation, as observed in Down syndrome and early AD. Increased Aβ1-42 levels and Aβ plaque deposition were not associated with selectively higher APP production; because of considerable intersubject variability, full-length APP isoforms detected on our Western blots were comparable in uninjured age-matched controls, because we cannot determine the magnitude of these changes. However, parallel assessment of Aβ levels in 2 positive control groups (postmortem tissue obtained from patients with AD and older controls) allows for translational comparison of the levels detected in TBI samples (previously unknown) to the range of reported human brain Aβ levels, and provides a standard for future TBI studies in humans.

In addition to known mechanisms involving diffuse axonal injury and direct trauma in TBI, elevation of soluble Aβ42 levels may have additional injurious effects, such as lowering neuronal resistance to injury or death. We saw no correlation of acute changes in soluble Aβ levels with outcome on the Glasgow Outcome Scale at 3 and 6 months. This could be because of modest sample size or lack of more sophisticated methods of outcome assessment. Alternatively, in long-term follow-up (years or decades), brain tissue accumulation of soluble Aβ monomers may lead to more progressive accrual of soluble Aβ oligomers, known to adversely affect cognitive function in mouse models, and insoluble Aβ fibrils. In this regard, chronic effects of postinjury increases in soluble Aβ42 and Aβ plaque formation on functional outcome and increased incidence of AD later in life remain to be examined in long-term survivors of severe TBI.

**CONCLUSIONS**

Higher brain tissue levels of soluble Aβ1-42 and the development of cortical Aβ plaques may predispose a subset of individuals with a brain injury to develop AD. Postinjury interventions that prevent or reduce such changes should be considered, especially in APOE ε4 carriers, as a therapeutic strategy to prevent progressive development of AD pathology and cognitive decline later in life.

Accepted for Publication: August 16, 2006.

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Financial Disclosure: None reported.

Funding/Support: This study was supported by grants NS30318 and NIA AG05133 from the National Institute of Neurological Disorders and Stroke.

Acknowledgment: We thank Ava Puccio, RN, MSN, for management of the Brain Trauma Research Center brain tissue bank, and Edward Dixon, PhD, Patrick Kochanek, MD, Larry Jenkins, PhD, and Steven Graham, MD, for their helpful discussions.
REFERENCES


Announcement

Online Submission and Peer Review System Available. The Archives of Neurology editorial office has introduced an online manuscript submission and peer review system developed by eJournalPress that will serve the needs of authors, reviewers, and editors. The new system went live on November 14, 2003. See http://archneur.ama-assn.org for more detailed information.