Association of Increased Cortical Soluble Aβ₄₂ 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 β-amyloid (Aβ) 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) Aβ peptides are posited to disrupt synaptic function and are associated with cognitive decline in Alzheimer disease. Changes in soluble Aβ levels and their relationship to Aβ plaque formation following TBI are unknown.

Objective: To quantify brain tissue levels of soluble Aβ peptides and their precursor protein in relation to Aβ plaque formation after TBI in humans.

Design: Surgically resected temporal cortex tissue from patients with severe TBI was processed for biochemical assays of soluble Aβ peptides with COOH-termini ending in amino acid 40 (Aβ₄₀) or 42 (Aβ₄₂) and Aβ precursor protein to compare patients with cortical Aβ 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 Aβ₄₂ but not Aβ₄₀; half of them were apolipoprotein E (APOE) ε4 allele carriers. The lowest Aβ levels were in 1 patient without plaques who was the only subject with an APOE ε2 allele. β-Amyloid precursor protein levels were comparable in the 2 TBI groups.

Conclusions: Selective increases in soluble Aβ₄₂ 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.

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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 Aβ1-40 and Aβ1-42 per gram of the wet weight of tissue. The APP was assayed by Western blot using an NH2-terminus–specific monoclonal 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, Aβ1-43, 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, Aβ1-43, 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
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 $\beta_{1-42}$ levels in patients with severe TBI and cortical plaques could explain the predominance of $\beta_{40}$ over $\beta_{42}$ plaques reported in human TBI autopsy series and in our biopsy immuno-histochemical study. $\beta_{1-42}$ is more prone to aggregation and may initiate plaque formation, as observed in Down syndrome and early AD. Increased $\beta_{1-42}$ levels and $\beta$ 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 between plaque-positive and plaque-negative TBI groups. Altered APP metabolic processing, not the effect of sex and APOE 4 allele, may explain the increased $\beta_{1-42}$ production in patients with cortical plaques. Accumulation of COOH-terminus APP fragments inside neuronal cell bodies and axons, demonstrated previously in this and other populations, is in accord with this idea. In addition, postinjury changes in $\beta$ aggregation dynamics or clearance mechanisms may increase $A\beta$ accumulation in brain tissue. For example, cerebrospinal fluid $\beta$ levels are decreased in patients with TBI, suggesting impaired clearance and increased retention of $\beta$ in brain tissue after injury. In accordance with this idea, a recent in vivo positron emission tomography imaging study using Pittsburgh compound B (an amyloid-binding compound), demonstrated that a decreased cerebrospinal fluid $\beta_{1-42}$ level (and not $\beta_{1-40}$) correlated with a greater Pittsburgh compound B retention (and therefore higher $\beta$ plaque load) during AD progression. Cerebrospinal fluid $\beta$ levels in patients with severe TBI and cortical plaques compared with patients with severe TBI without cortical plaques remain to be determined.

Levels of $\beta$ after injury could also be influenced by patients’ demographic factors. In the current study, both female patients and APOE e4 carriers were prevalent in the plaque-positive group. The effect of sex on $\beta$ levels and deposition after TBI is not known. As confirmed in the present study, the frequency of APOE e4 is high in patients with TBI with $\beta$ plaques; the presence of this allele is also associated with a 10-fold increased risk for developing AD after TBI. Although the APOE e4 allele and female sex were associated with higher soluble $\beta$ levels overall, this difference was not statistically significant in the present study.

While our data demonstrate that plaque formation after TBI is associated with greater soluble $\beta_{1-42}$ levels, we cannot determine the magnitude of these changes relative to uninjured age-matched controls, because fresh biopsy tissue from such subjects is not available. However, parallel assessment of $\beta$ levels in 2 positive control groups (postmortem tissue obtained from patients with AD and older controls) allows for transla-

tion of the levels detected in TBI samples (previously unknown) to the range of reported human brain $A\beta$ 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 $\beta_{1-42}$ levels may have additional injurious effects, such as lowering neuronal resistance to injury or death. We saw no correlation of acute changes in soluble $\beta$ 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 $A\beta$ monomers may lead to more progressive accrual of soluble $A\beta$ oligomers, known to adversely affect cognitive function in mouse models, and insoluble $A\beta$ fibrils. In this regard, chronic effects of postinjury increases in soluble $A\beta_40$ and $A\beta$ 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 $\beta_{1-42}$ and the development of cortical $A\beta$ 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 e4 carriers, as a therapeutic strategy to prevent progressive development of AD pathology and cognitive decline later in life.

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