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 Aβ₁₋₄₂ but not Aβ₁₋₄₀; 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 Aβ₁₋₄₂ 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.

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The molecular mechanism of Aβ plaque formation after TBI in humans is not known, but likely involves altered Aβ precursor protein (APP) production and/or metabolism, which occurs in animal TBI models. After TBI in humans, accumulation of APP fragments (including Aβ) in damaged axons and plaques has been observed using immunohistochemical techniques. Reduced levels of soluble Aβ have been detected in cerebrospinal fluid, suggesting that reduced Aβ clearance may contribute to increased Aβ levels in brain tissue. To date, there have been no quantitative biochemical assessments of Aβ peptides and APP in human brain tissue after TBI. Given the potential synaptotoxic effects of soluble Aβ peptides and plaque-associated fibrils in AD, understanding postinjury changes in soluble Aβ could provide insight into the pathologic sequelae of MYLOID PLAQUES, COMPOSED PREDOMINANTLY OF β-AMYLOID (Aβ) PEPTIDES WITH COOH-TERMINI ENDING IN AMINO ACID 40 (Aβ₄₀) AND 42 (Aβ₄₂), ACCUMULATE PROGRESSIVELY IN THE BRAINS OF PATIENTS WITH ALZHEIMER DISEASE (AD). THE EXACT CAUSE OF THESE LESIONS IS NOT CLEAR. DIFFUSE DEPOSITS OF Aβ₄₀ ARE POSITED TO BE AN INITIAL FORM OF Aβ PLAQUES; IN INDIVIDUALS WITH NO AD DEMENTIA, THEY ARE RARELY DETECTED BEFORE AGE 50 YEARS AND ARE CONSIDERED INDICATIVE OF “PATHOLOGICAL AGING” OR PRECLINICAL AD. IN CONTRAST, THERE HAVE BEEN CONSISTENT NEUROPATHOLOGIC REPORTS OF DIFFUSE CORtical Aβ PLAQUES IN 30% TO 38% OF PEOPLE WITH TRAUMATIC BRAIN INJURY (TBI) REGARDLESS OF THEIR AGE. TOGETHER WITH EPIDEMIOLOGICAL STUDIES, THIS NEUROPATHOLOGIC EVIDENCE SUGGESTS THAT TBI IS AN ENVIRONMENTAL RISK FACTOR FOR THE DEVELOPMENT OF AD.
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. The APP was assayed using Western blot analysis of APP revealed 2 major bands, one at 115 and 105 kd, corresponding to expected molecular weights of 116 and 106 kDa, respectively. The other band was prominent in all patients with TBI and cortical plaques (plaque positive) (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 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, Aβ1-42 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...
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 between plaque-positive and plaque-negative TBI groups. Altered APP metabolic processing, not the focus of the present study, may contribute to greater plaque formation. Because of considerable intersubject variability, we cannot determine the magnitude of these changes in life.

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.

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