Increase of Brain Oxidative Stress in Mild Cognitive Impairment: A Possible Predictor of Alzheimer Disease

Domenico Praticò, MD; Christopher M. Clark, MD; Feyan Liu, MD; Virginia Y.-M. Lee, PhD; John Q. Trojanowski, MD, PhD

Background: The isoprostane 8,12-iso-iPF₃₋₁₋₁₋₁, a specific marker of in vivo lipid peroxidation, is increased in Alzheimer disease (AD). The pathological changes associated with AD have a long silent phase before the appearance of clinical symptoms. Several studies have shown that AD is preceded by a prodromal phase characterized by mild cognitive impairment (MCI).

Objective: To investigate levels of this biomarker in subjects with MCI.

Design and Main Outcome Measures: Using gas chromatography–mass spectrometry analysis, we measured 8,12-iso-iPF₃₋₁₋₁₋₁ levels in urine, plasma, and cerebrospinal fluid of patients with AD, subjects with MCI, and cognitively normal elderly subjects.

Setting and Patients: Subjects attending the Memory Disorders Clinic.

Results: We found significantly higher 8,12-iso-iPF₃₋₁₋₁₋₁ levels in cerebrospinal fluid, plasma, and urine of subjects with MCI compared with cognitively normal elderly subjects.

Conclusions: These results imply that individuals with MCI have increased brain oxidative damage before the onset of symptomatic dementia. Measurement of this isoprostane may identify a subgroup of patients with MCI with increased lipid peroxidation who are at increased risk to progress to symptomatic AD.

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Alzheimer disease (AD) is the most common cause of dementia in the elderly and may have a long stage of neuropathological changes and cognitive decline before it is diagnosed.1 An increasing number of studies clearly indicate that the onset of AD is typically preceded by an interim phase known as mild cognitive impairment (MCI).2 3 In its amnestic version, MCI is characterized primarily by a memory deficit without clinically meaningful functional impairment.4 Typically, the onset of MCI is marked by a measurable memory loss that is abnormal for an individual's age and education and is corroborated by an informant.5

Non-Alzheimer dementia may have a prodromal phase that differs in its cognitive profile. Despite potential heterogeneity of an MCI diagnosis, recent data have suggested that it is associated with up to a 50% probability of progressing to symptomatic AD within a 4-year period.6 Thus, the rate of progression from MCI to AD is approximately 12% per year, supporting the concept that MCI, at least in part, represents the prodromal stage of AD.7

For these reasons it is important to identify and biochemically characterize these patients in the earliest phase of their illness, since it is in this phase that interventional therapy should have the greatest potential to slow down disease progression. Factors associated with a more rapid progression from MCI to AD include the presence of an apolipoprotein E ε4 allele and a small hippocampus as measured on magnetic resonance imaging.8 9

We have recently reported that isoprostanes (iPs), sensitive and specific markers of in vivo lipid peroxidation,10 are increased in cerebrospinal fluid (CSF), blood, and urine of patients with a clinical diagnosis of AD. These levels were highly correlated with other biomarkers of AD pathology and with the severity of the disease.11 Since individuals with MCI are believed to be at high risk to progress to a clinical diagnosis of AD, we investigated whether they, like patients with AD, have high levels of this marker.

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SUBJECTS AND METHODS

SUBJECTS

This study was reviewed and approved by the institutional review board of the University of Pennsylvania, Philadelphia. Subjects were recruited from the University Alzheimer’s Disease Center Memory Disorders Clinic (MDC) between May 1, 1998, and February 28, 2001. Informed consent was obtained from all participants and their caregivers.

The clinical diagnosis of probable AD was based on the National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer’s Disease and Related Disorders Association criteria.12 Criteria for the diagnosis of MCI were the following: (1) memory complaint documented by the patient or collateral source; (2) scores on standardized cognitive testing that were 2 SDs below age- and education-adjusted normal values in 1 domain or 1 SD below normal in 2 domains; (3) Clinical Dementia Rating of 0.5 or more; and (4) no symptoms of dementia based on a clinical examination and an extensive interview with a knowledgeable informant.

As part of their routine cognitive assessment, all patients underwent the Consortium to Establish a Registry for Alzheimer’s Disease psychometric battery, which assesses memory, language, and constructional praxis,13 plus additional tests of memory (Logical Memory I and II), visual memory (Constructional Praxis recall and recognition), and attention (Digit Symbol Substitution). Additional measures included the Mini-Mental State Examination14 and Dementia Severity Rating Scale.15 Routine laboratory studies, including magnetic resonance imaging, were performed to exclude other causes of cognitive impairment.

To the best of our knowledge, no subject with familial AD was included in the study. Subjects were also excluded if they had evidence of an acute infectious or inflammatory disease, chronic hepatic disease, chronic obstructive pulmonary disease, alcoholism, or cancer, since these conditions may affect F2-iPs biosynthesis.10 Urine and blood samples were obtained from 50 patients with AD, 33 with MCI, and 40 control subjects. In addition, within 2 weeks, CSF was obtained from 28 of the 50 patients with AD, 17 of the 33 subjects with MCI, and 18 of the control subjects. A second urine sample was collected at this time. The elderly control subjects were recruited from a cohort of cognitively normal individuals followed up by the Alzheimer’s Disease Center and from cognitively normal spouses of patients with AD or MCI attending the Memory Disorders Clinic. During the follow-up period, 5 subjects with MCI progressed to AD.

RESULTS

Table 1 represents the clinical characteristics of the population studied. There was no significant difference among the 3 groups with respect to age and years of education (Table 1), plasma cholesterol level, triglycerides level, diabetes, or smoking habit (not shown). Similarly, there was no difference among the 3 groups with respect to diet, weight, and vitamin intake (not shown). Apolipoprotein E ε2, ε3, and ε4 allele distribution in the patients with AD and MCI are shown in Table 1. Patients with probable AD had urinary 8,12-iso-iPF(2ω)-VI levels significantly greater than that of control subjects (4.6±0.2 vs 1.5±0.1 ng/mg of creatinine) (P<.001) and patients with MCI (3.6±0.3 vs 1.5±0.1 ng/mg of creatinine) (P=.01) (Figure, A). Patients with MCI had higher urinary 8,12-iso-iPF(2ω)-VI levels than control subjects (3.6±0.3 vs 1.5±0.1 ng/mg of creatinine) (P<.001). Similarly, there was a signifi-
cant difference in plasma isoprostane levels between patients with AD (0.61±0.03 ng/mL) and MCI (0.44±0.03 ng/mL) (P/H11021.03) and between patients with MCI and control subjects (0.19±0.01 ng/mL) (P/H11021.001) (Figure, B). There were no significant clinical or demographic differences in age, symptom severity, or disease duration between those members who did or did not undergo lumbar puncture in each of the 3 groups. Likewise, there was no difference in the urinary 8,12-iso-iPF2_{α, α}-VI levels measured at the time of lumbar puncture compared with the initial sample (data not shown). A significant increase in CSF 8,12-iso-iPF2_{α, α}-VI levels in both the AD and MCI groups compared with the control group was found (P/H11021.001) (Figure, C). In particular, patients with AD had a level of 68±6.3 pg/mL compared with 44±7.1 pg/mL for patients with MCI (P=0.03) and 15±1.2 pg/mL for control subjects. There was a direct correlation between CSF and urinary levels of 8,12-iso-iPF2_{α, α}-VI and between CSF and plasma 8,12-iso-iPF2_{α, α}-VI levels. The coefficient of correlation (r^2) for each was 0.55 and 0.64, respectively (both P<.001).

As would be consistent with the selection criteria, the patients with AD had impaired cognitive function as shown by the Mini-Mental State Examination and Dementia Severity Rating Scale assessments (Table 1). By contrast, subjects with MCI performed slightly more poorly on these measures than the controls subjects but were superior to the patients with AD. The CSF tau protein level was elevated, whereas the percentage ratio between CSF Aβ_{1-40} and Aβ_{1-42} was lower in patients with AD than in patients with MCI and matched control subjects (Table 2). In particular, patients with AD had significantly higher CSF tau levels and lower percentage of Aβ_{1-42} than control subjects. By contrast, only CSF tau levels, but not the percentage of Aβ_{1-42}, were significantly higher in AD than in MCI. Moreover, no statistically significant difference was observed between patients with MCI and matched control subjects for both variables (Table 2). Finally, no difference in 8,12-iso-iPF2_{α, α}-VI levels was found between patients homozygous for apolipoprotein E e4 allele and those with 1 or no e4 allele (not shown).

In this study we show that patients who meet standardized clinical criteria for MCI have increased CSF,

Table 1. Demographic Characteristics of the Population Studied*

<table>
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<tr>
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<th>AD Group (n = 50)</th>
<th>MCI Group (n = 33)</th>
<th>Control Subjects (n = 40)</th>
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<td>100</td>
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*AD indicates Alzheimer disease; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; DSRS, Dementia Severity Rating Scale; NT, not tested; and NA, not applicable.
†P<.001 vs AD.
plasma, and urinary levels of 8,12-iso-iPF2α-VI, a reliable marker of in vivo lipid peroxidation.10 At the same time, we confirm our previous finding that subjects with AD have higher levels of this marker. Patients with MCI constitute an important group to study for both theoretical and practical reasons. This is a group with well-characterized clinical diagnostic criteria.4,5 In particular, most of these patients have a memory impairment that is beyond normal limits and yet are functioning independently. These individuals are at high risk to progress to meet clinical criteria for AD and do so at a rate of 10% to 12% per year,6,7 and therefore it is important to identify them as they represent an ideal target for preventive strategies.

A consistent body of evidence clearly indicates that oxidative stress is increased in brains of patients with AD as well as in living patients with probable AD.19,20 Previously, our group reported that patients with AD have elevated levels of 8,12-iso-iPF2α-VI in CSF, plasma, and urine when compared with control subjects,11 suggesting that lipid peroxidation is an early event in AD and that measurement of this isoprostane could be a sensitive marker of brain oxidative damage. This observation was confirmed by Tupper et al,21 who measured urinary levels of another distinct F2-isoprostane, iPF2α-III, in patients with AD. However, another group, by measuring total F2-isoprostane levels, failed to find a difference in urine and plasma levels in AD and found such a difference only in CSF.21 It is possible that methodologic differences explain these contrasting results.

Our finding that levels of this specific isoprostane are significantly elevated in subjects who meet clinical criteria for MCI adds further support to the notion that oxidative stress is an early pathological change in AD.

Since the earliest neurologic changes of AD begin well before a clinical diagnosis can be made, there is considerable benefit to the identification of an easily obtainable biological marker that could identify patients with MCI who are at highest risk to progress to AD.22,23 Previous works demonstrated that apolipoprotein E ε4 carrier status and the identification of mild memory impairment might predict who is likely to progress to AD.5,8,25 Magnetic resonance imaging volumetric measurements of the hippocampal formation9 and CSF tau and Aβ1-42 levels may also be useful in this task.26

In accordance with previous reports, we found that, among the 3 groups studied, patients with AD had the highest values for CSF tau and the lowest percentage ratio between Aβ1-40 and Aβ1-42, but no significant difference was observed between subjects with MCI and control subjects. By contrast, subjects with MCI had CSF 8,12-iso-iPF2α-VI levels significantly higher than those of elderly control subjects. Considering that CSF tau and Aβ levels are thought to be markers of AD neuropathologic changes and disease progression,17 this observation would suggest that brain oxidative damage is an early event in the development of AD-like pathology. Remarkably, in our study we found that subjects with MCI are different from elderly control subjects with respect only to this marker of oxidative stress. This suggests that measurement of 8,12-iso-iPF2α-VI may provide a reliable biomarker of brain oxidative damage that could help in identifying, together with hippocampus volumetric analyses and apolipoprotein E ε4 allele analysis, subjects with MCI who are at higher risk to develop to AD. Parenthetically, 5 of the subjects with MCI, all with high 8,12-iso-iPF2α-VI levels, converted to AD during the follow-up. However, future longitudinal studies are needed to address this hypothesis.

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Author contributions: Study concept and design (Drs Pratico and Clark); acquisition of data (Drs Pratico, Clark, and Liun); analysis and interpretation of data (Drs Pratico, Clark, Lee, and Trojanowski); drafting of the manuscript (Drs Pratico and Liun); critical revision of the manuscript for important intellectual content (Drs Pratico, Clark, Lee, and Trojanowski); statistical expertise (Drs Pratico, Clark, Liun, and Trojanowski); study supervision (Drs Pratico and Clark).

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REFERENCES


CME Announcement

CME Hiatus: July Through December 2002. CME from JAMA/ARCHIVES will be suspended between July and December 2002. Beginning in early 2003, we will offer a new online CME program that will provide many enhancements:

- Article-specific questions
- Hypertext links from questions to the relevant content
- Online CME questionare
- Printable CME certificates and ability to access total CME credits

We apologize for the interruption in CME and hope that you will enjoy the improved online features that will be available in early 2003.

Correction

Errors in Byline, Author Affiliations, and Author Contributions. In the Original Contribution by Praticò et al titled “Increase of Brain Oxidative Stress in Mild Cognitive Impairment: A Possible Predictor of Alzheimer Disease,” published in the June issue of the ARCHIVES (2002;59:972-976), an author was inadvertently omitted from the byline, author affiliations, and author contributions. The byline on page 972 should have read as follows: “Domenico Praticò, MD; Christopher M. Clark, MD; Feyan Liun, MD; Joshua Rokach, PhD; Virginia Y.-M. Lee, PhD; John Q. Trojanowski, MD, PhD.” The author affiliations on page 972 should have appeared as follows: “From the Center for Experimental Therapeutics and Department of Pharmacology (Drs Praticò and Liun), Department of Neurology (Dr Clark), Center for Neurodegenerative Disease Research (Drs Lee and Trojanowski), and Alzheimer’s Disease Center (Drs Clark, Lee, and Trojanowski), University of Pennsylvania School of Medicine, Philadelphia; and the Claude Pepper Institute for Aging and Therapeutic Research, Florida Institute of Technology, Melbourne (Dr Rokach).” The author contributions on page 975 should have read as follows: “Study concept and design (Drs Praticò and Clark); acquisition of data (Drs Praticò, Clark, and Liun); analysis and interpretation of data (Drs Praticò, Clark, Rokach, Lee, and Trojanowski); drafting of the manuscript (Drs Praticò and Liun); critical revision of the manuscript for important intellectual content (Drs Praticò, Clark, Rokach, Lee, and Trojanowski); statistical expertise (Drs Praticò, Clark, Liun, and Trojanowski); administrative, technical, and material support (Dr Rokach); and study supervision (Drs Praticò and Clark).”

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