BRIEF REPORT

Rate of progression in Alzheimer's disease correlates with coated-platelet levels—a longitudinal study

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Coated-platelets represent a subset of platelets produced by activation with both collagen and thrombin that retain full-length amyloid precursor protein on their surface. In our initial cross-sectional study, coated-platelet levels correlated inversely with disease severity in Alzheimer's disease (AD). Higher levels were observed in the early stage compared with the advanced stage of the disease. In this longitudinal study, we investigated for the first time the relationship between initial coated-platelet levels and disease progression in individuals with AD. Coated-platelet levels were assayed in 25 patients with AD who where then clinically monitored for 2 years. A significant linear correlation ($r = 0.47$, $P = 0.017$) was detected between the initial coated-platelet levels and disease progression measured in the Mini-Mental State Examination score. The most severe decline was noted in individuals with the highest initial coated-platelet production. These findings support our previous observations from cross-sectional studies and suggest the need for additional study of coated–platelets as a link to the sequence of events leading to the development of AD. (Translational Research 2008;152:99–102)

Abbreviations: ACD = acid citrate dextrose; AD = Alzheimer's disease; APP = amyloid precursor protein; MCI = mild cognitive impairment; MMSE = Mini-Mental Status Examination; MRI = magnetic resonance imaging; PRP = platelet-rich plasma; SD = standard deviation

Coated-platelets represent a subset of activated platelets observed during dual-agonist stimulation with collagen and thrombin.[1] We have previously demonstrated that, in sharp contrast to platelets activated with a single agonist, coated-platelets retain full-length amyloid precursor protein (APP) on their surface.[2] In addition, we have shown that coated-platelet levels are increased in early stage Alzheimer's disease (AD) patients compared with AD patients with advanced disease and normal elderly controls.[2] In a previous cross-sectional study investigating coated-platelet production in AD patients in various stages of disease severity, we observed a significant linear relationship ($r^2 = 0.26$; $P = 0.001$) between coated-platelet levels and the Mini-Mental State Examination (MMSE) score.[3] Most recently, we determined that coated-platelet levels are increased in patients with amnestic versus nonamnestic mild cognitive impairment (MCI),[4] a finding of significant clinical relevance since amnestic MCI patients are considered to be at increased risk for progression to AD, whereas nonamnestic MCI patients are more likely to progress to non-AD dementias or may simply be part of normal cognitive aging.[5]

To date, no investigation has been conducted to explore the relationship between initial coated-platelet

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AT A GLANCE COMMENTARY

Background

Coated-platelets represent a subset of platelets produced by activation with both collagen and thrombin. We previously demonstrated that 1) amyloid precursor protein is retained exclusively by coated-platelets, 2) coated-platelet levels correlate with Alzheimer's disease (AD) severity in cross-sectional studies, and 3) increased coated-platelet synthesis is present in amnestic mild cognitive impairment (MCI) as compared with nonamnestic MCI patients.

Translational Significance

This article reports the first prospective investigation of a relationship between coated-platelet levels and disease progression in individuals with AD and represents an example of translational research by bridging basic science (coated-platelets) with clinical science (AD).

levels and disease progression in individuals with AD. The current study investigated this relationship in a sample of AD patients that were clinically monitored for disease progression over 2 years. Based on our previous data,[2,3] we hypothesized that initial coated-platelet levels will correlate positively with the rate of disease progression.

METHODS

Study subjects. This study was carried out according to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Oklahoma and the Veterans Affairs Research and Development Committee. Informed consent was obtained for all participants prior to inclusion in the study. Because individuals with dementia are considered a protected group as defined by the Institutional Review Board of the University of Oklahoma rules and regulations, each patient was accompanied by the next of kin during the initial consenting and enrollment and during any subsequent visits.

Twenty-five patients were recruited through the Center for Alzheimer's and Neurodegenerative Disorders at the Oklahoma City Veterans Affairs Hospital. All patients fulfilled clinical criteria for probable AD[6] and had a complete diagnostic evaluation for dementia, including brain magnetic resonance imaging (MRI) scans. MMSE[7] was assessed at the time of enrollment and repeated after 2 years for all subjects. The presence of white matter disease at the time of enrollment was assessed based on published criteria using MRI imaging studies[8] by a rater (E.D.R.) who was not aware of the MMSE score or the coated-platelet level of the given patient. The disease progression for each individual was measured by subtracting the 2-year repeat MMSE from the initial MMSE. All patients received treatment with cholinesterase inhibitors at the time of study enrollment.

Exclusion criteria included a diagnosis of dementia other than AD or a history of stroke, transient ischemic attack, or acute coronary syndrome.[1] Additional exclusion criteria included current smoking and certain classes of medications such as selective serotonin reuptake inhibitors, HMG-CoA reductase inhibitors, or antiplatelet agents, as these factors may influence coated-platelet levels.[1,9]

Coated-platelet assay. After obtaining informed consent, 5 mL of blood was drawn into a plastic syringe containing 0.5 mL of acid citrate dextrose (ACD), and platelet-rich plasma (PRP) was prepared as described. Coated-platelets were assayed as previously described[9] with 1 μL of PRP in a 100-μL assay with the following reagents (final concentrations): 1.0-μg/mL biotin-fibrinogen, 0.4-mmol/L gly-pro-arg-pro, 500-ng/mL convulxin, 0.5-U/mL bovine thrombin, 2-mmol/L $CaCl_2$, 1-mmol/L $MgCl_2$, 150-mmol/L NaCl, and 10-mmol/L N-(2-hydroxyethyl)-piperazine-N'-(4-butanesulfonic acid) (HEPES), pH 7.5. After 5 min at 37°, 0.8 μg of phycoerythrin-streptavidin and 0.5 μg of FITC-abciximab were added. After an additional 5 min at 37°, the reaction was stopped with 0.2 mL of 1.5% (wt/vol) formalin in 150-mmol/L NaCl, 10-mmol/L HEPES, and pH 7.5. The percentage of abciximab-positive events (platelets) with bound biotin-fibrinogen was quantitated by flow cytometry.[1,9] The coated-platelet levels are reported as percentages of the total platelets.[1,9] The individuals performing the coated-platelet assay were not aware of the clinical diagnosis corresponding to the blood sample analyzed.

Statistical analyses. Data were analyzed using SAS (SAS System for Windows, ver. 9.1.3; SAS Institute Inc., Cary, NC) and SPSS (SPSS for Windows, rel. 12.0.0; SPSS Corporation, Chicago, Ill). Correlational analyses with significance set at $P \leq 0.05$ were performed to explore the relationship between coated-platelet levels at the time of enrollment and disease progression measured in MMSE points over the period of follow-up. The impact of age, level of education, and white matter changes on the observed relationship between coated-platelet levels and disease progression was also examined. The sample size was calculated to achieve a 70% power to detect a significant ($P < .05$ and $r = 0.35$) linear correlation between coated-platelet levels and disease progression, based on our previous data and on expected disease progression in AD. Standard deviations (SDs) are provided when means are presented in the text (see the Results section).

RESULTS

All individuals in the study were male as a result of the composition of the United States armed forces during the time these veterans served. The patients' mean age was 76.4 $\pm$ 8.0 years (mean $\pm$ SD, range 54−85, n = 25), and their level of education was 12.2 $\pm$ 2.2 years (range 7−16). The initial MMSE score was