Amyloid Precursor Protein in Platelets of Patients With Alzheimer Disease

Effect of Acetylcholinesterase Inhibitor Treatment

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Background: Amyloid precursor protein (APP) forms with apparent molecular weights of 130, 110, and 106 kd are present in human platelets. It has been demonstrated that Alzheimer disease (AD) is specifically associated with a decreased APP forms ratio in platelets.

Objective: To investigate whether acetylcholinesterase (AChE) inhibitor treatment modifies the ratio of platelet APP forms in patients with AD.

Patients and Methods: From a large sample of patients with probable AD, 30 with mild to moderate AD were selected. Each patient underwent a clinical evaluation including the Mini-Mental State Examination (MMSE) and platelet APP forms analysis at baseline and after 30 days. During this interval, 20 of 30 patients with AD were treated with donepezil hydrochloride (5 mg/d), a piperidine phosphate–based cholinesterase inhibitor. Platelets were subjected to Western blot analysis using monoclonal antibody (22C11). The ratio between the immunoreactivity of the higher-molecular-weight APP form (130 kd) and the lower forms (106 and 110 kd) was measured.

Results: All patients taking donepezil completed the 30 days of treatment without adverse effects. The platelet APP forms ratio at baseline did not differ between the 2 AD groups (mean ± SD optical density ratio: untreated AD, 0.47 ± 0.12; treated AD, 0.38 ± 0.18), whereas a significant difference was found at follow-up (mean ± SD optical density ratio: untreated AD, 0.45 ± 0.17; treated AD, 0.77 ± 0.29; P < .001). A significant improvement in MMSE scores in treated AD patients was observed from baseline (16.9 ± 3.8) to 30 days (18.9 ± 4.42) (P < .009, 30 days vs baseline), but no significant correlation was found in treated AD patients between MMSE score improvement and APP forms/ratio increase (P = .09).

Conclusions: Administration of AChE inhibitors increases the ratio of APP forms in platelets of patients with AD, suggesting a potential effect of AChE inhibitors on APP trafficking or processing in a peripheral cell.

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Subjects and Methods

Subjects

Patients with probable AD and controls were recruited from the Neurological Clinic of Brescia, Brescia, Italy, and from the Centro Alzheimer of Passirana-Rho, Milan, Italy. The study was conducted in accordance with local clinical research regulations. Written informed consent was obtained from the patient and the caregiver. All participants underwent medical, epidemiologic, and neuropsychologic assessments. Additional diagnostic testing included neuroimaging (computed tomography or magnetic resonance imaging), blood tests, and other evaluations as needed. A diagnosis of dementia was made according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). A diagnosis of probable AD was based on National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association criteria. Patients were followed up for at least 1 year before being included in the study. Dementia severity was measured through the Clinical Dementia Rating Scale. Mini-Mental State Examination (MMSE) scores at the time of sampling were also recorded.

The following exclusionary criteria for the AD group were designed to ensure that participants had probable AD as the cause of their dementia: (1) major depressive disorder, bipolar disorder, schizophrenia, substance use disorder, or mental retardation according to the criteria of the DSM-IV; (2) cerebrovascular disorders, hydrocephalus, and intracranial mass, documented by computed tomography or magnetic resonance imaging within the past 12 months; (3) abnormalities in serum folate and vitamin B12 levels, syphilis serologic findings, or thyroid hormone levels; (4) a history of traumatic brain injury or another neurologic disease (eg, Parkinson disease, Huntington disease, or seizure disorders); and (5) significant medical problems (eg, poorly controlled diabetes or hypertension; cancer within the past 5 years; or clinically significant hepatic, renal, cardiac, or pulmonary disorders). To avoid potential pharmacologic confounding effects on platelet physiologic findings, patients and controls taking psychotropic agents, nootropic drugs, antiplatelet agents, anticoagulants, corticosteroids, and serotoninergic drugs entered the study only after being drug free for at least 14 days before blood sample collection and platelet preparation. Concomitant treatment with these drugs was not allowed during the study.

Study Design

This was a 4-week, longitudinal, open study conducted in 2 medical centers. Of 30 consecutive patients with AD, 20 received no drug treatment (AD-n) and 20 were treated with donepezil, 5 mg/d (AD-d). Treatment group status was assigned by patient eligibility to receive AChE inhibitors based on the presence of well-known contraindications (ie, supraventricular cardiac conditions, ulcer disease, history of seizure, history of asthma, or obstructive pulmonary disease). Patients in the AD-d group received a single dose of donepezil each evening. All patients were investigated at baseline and after 30 days. At each session, AD-n and AD-d patients and 10 controls were subjected to a clinical evaluation, including an MMSE and a venipuncture for platelet sample collection.

Platelet Preparation

Blood samples were drawn from fasting participants between 9 and 10 AM. Patient information and case diagnoses were unknown to the laboratory investigators who received and analyzed the samples. A blood sample (27 mL) was taken, with the tourniquet carefully released immediately after its application, from a vein in the antecubital fossa using a 19-gauge needle and collected into 3 mL of 3.8% sodium citrate (in the presence of glucose, 136 mmol/L). Each sample was mixed gently and centrifuged at 200g for 10 minutes to separate platelet-rich plasma within 30 minutes of blood drawing. Platelet-rich plasma was separated from the blood pellet by means of a plastic pipette, with aspiration of the buffy coat avoided. Platelets were then collected by further centrifugation at 500g for 20 minutes and washed, and the platelet pellet was stored at −80°C until used.

Immunoblot experiments were performed with monoclonal antibody 22C11 as described elsewhere. Results are expressed as the ratio between the optical density of the upper (130-kd) and lower (106- and 110-kd) 22C11 immunoreactive bands. The ratio was determined for each individual from at least 3 replications.

Statistical Analysis

Quantitative Western blot analysis was performed by means of computer-assisted imaging (Imaging System-Quantity One; Bio-Rad, Hercules, Calif). Results were averaged and are expressed as mean±SD. Clinical and laboratory results before and after treatment were assessed using paired t tests. Differences were considered statistically significant at P<.05 (2-tailed).

Hyperactivity have been implicated as an explanation for the early memory impairment. On this ground, AChE inhibitors were introduced as therapeutic tools to restore the amount of acetylcholine available in the synaptic cleft. Evidence that the use of AChE inhibitors produces a significant improvement in cognitive performance might exert a neuroprotective role by modulating acetylcholine receptors and in turn enzymes responsible for APP metabolism. However, most of these results have been derived through short-term treatment in vitro or from animals treated long term. In fact, in vivo data on human cells are lacking, leaving still unanswered the question about the real efficacy to modify in vivo APP metabolism through AChE inhibitor use. The aim of this study was to evaluate the effect of AChE inhibitor drug therapy on APP metabolism in vivo...
using platelets as a peripheral model. We longitudinally investigated the APP forms ratio in platelets of patients with AD treated for 1 month with donepezil hydrochloride (5 mg/d) and untreated patients with AD, and we compared these groups with the platelet APP forms ratio in control subjects using platelets as a peripheral model. We longitudinally investigated the APP forms ratio in platelets of patients with AD treated for 1 month with donepezil hydrochloride (5 mg/d) and untreated patients with AD, and we compared these groups with the platelet APP forms ratio in control subjects.

## RESULTS

### BASELINE

Demographic and clinical characteristics of the 2 patient groups (AD-n and AD-d) and the control group are shown in the Table.

![Representative Western blot analysis performed with 22C11 on whole platelet homogenate showing amyloid precursor protein forms at baseline (T=0) and after 30 days (T=30) obtained from a patient with untreated Alzheimer disease (AD-n; lanes 1 and 2), a patient with treated AD (AD-d; lanes 3 and 4), and a control subject (lanes 5 and 6). The arrows indicate the position of the amyloid precursor protein forms and their apparent molecular weight.](image)

Figure 1. Representative Western blot analysis performed with 22C11 on whole platelet homogenate showing amyloid precursor protein forms at baseline (T=0) and after 30 days (T=30) obtained from a patient with untreated Alzheimer disease (AD-n; lanes 1 and 2), a patient with treated AD (AD-d; lanes 3 and 4), and a control subject (lanes 5 and 6). The arrows indicate the position of the amyloid precursor protein forms and their apparent molecular weight.

Both AD patient groups had significantly impaired MMSE scores (AD-d, 16.9±4.1; AD-n, 17.4±6.1) at baseline compared with controls (28.3±1.6; AD-d vs controls and AD-n vs controls; P<.001). The cumulative quantitative analysis is shown in Figure 2. Donepezil treatment determined a 2-fold increase in the APP forms ratio in the AD-d group (P<.001), whereas the APP forms ratio remained unchanged from baseline in the AD-n group.

A significant improvement in MMSE scores was observed from baseline (16.9±3.8) to 30 days (18.9±4.42) (P<.009, 30 days vs baseline) in the AD-d group but not in the AD-n group (17.4±6.1 and 18.1±5.2, respectively; P=.2, 30 days vs baseline). However, no significant correlation was found between the improvement in MMSE scores and the APP forms ratio changes in the AD-d group.

In the present study, the effect of 30 days of donepezil treatment, a piperidine-based cholinesterase inhibitor,
Results of our study, however, in agreement with experimental data mostly derived by in vitro experiments or animal models, suggest that AChE inhibitors, at a dosage commonly administered in clinical practice as therapeutic for AD, might modify the concentration of APP forms in human platelets, rescuing the values of the ratio of APP forms to control levels. Whether this effect directly affects a fundamental feature of AD pathogenesis is still a matter of study.

The significant effect on the APP forms ratio exerted by donepezil therapy suggests that this peripheral marker might be useful to monitor not only disease progression but also pharmacologic manipulations. Our data, in addition, strongly support the evidence that sample selection needs to be strictly defined because peripheral markers are susceptible to biological manipulation at different levels.

Thirty days of donepezil treatment improved MMSE scores in patients with AD. However, there was no relation between cognitive and APP forms ratio changes. Such negative findings might be due to the small patient sample and the short interval of evaluation; pharmacologic effect on cognitive function is also associated with different factors, such as level of cholinergic damage, genetic factors, or sex, whose effect on biological variables is less likely.

Understanding of the molecular mechanism responsible for rescuing platelet APP levels in patients with AD after donepezil treatment still needs further investigation.

Although it is known that platelets express the repertoire of enzymes necessary for APP processing and AChE, it is difficult to ascribe the donepezil effect to a generic increase in the concentration of acetylcholine in biological fluids, which might in turn activate through its receptor(s) a biochemical cascade capable of affecting APP processing.

More likely, the effect of donepezil involves a direct link between AChE and α-secretase or APP in the peripheral compartment. Recently, it was suggested that AChE and α-secretase might cluster in plasma membrane. Thus, the interaction of donepezil with AChE might prime a conformational modification of the enzyme that reflects a modification in α-secretase activity. This might restore a correct balancing in metabolism and redistribution of APP forms in the membrane. Alternatively, donepezil treatment might indirectly affect APP trafficking either in platelets or in megakaryocytes, thus making the protein more prone to membrane insertion and to α-secretase activity. Indeed, donepezil might affect glycosylation of APP forms that, in turn, might affect its processing. In addition, to further clarify this in vivo effect of donepezil on platelet APP forms, it would be of interest to examine different time courses of the pharmacologic treatment to determine whether donepezil is affecting platelets or megakaryocytes and to establish the stability and the maximum effect. Experiments are in progress in our laboratory to elucidate this aspect.

In conclusion, the results of the present study demonstrate that the use of AChE inhibitors such as donepezil modifies APP processing in the platelets of patients with AD. The platelet APP forms ratio therefore holds the po-
potential to be a peripheral marker that might be helpful as a tool for studying mechanisms underlying APP metabolism and for the assessment of pharmacologic effect.

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REFERENCES


