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Short Communication

Cognitive Stimulation Modulates Platelet Total Phospholipases A_2 Activity in Subjects with Mild Cognitive Impairment

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Abstract. We evaluated the effect of cognitive stimulation (CS) on platelet total phospholipases A_2 activity ($tPLA_2A$) in patients with mild cognitive impairment (MCI_P). At baseline, $tPLA_2A$ negatively correlated with Mini-Mental State Examination score (MMSE_s): patients with MMSE_s <26 (Subgroup 1) had significantly higher activity than those with MMSE_s ≥26 (Subgroup 2), who had values similar to the healthy elderly. Regarding CS effect, Subgroup 1 had a significant $tPLA_2A$ reduction, whereas Subgroup 2 did not significantly changes after training. Our results showed for the first time that $tPLA_2A$ correlates with the cognitive conditions of MCI_P, and that CS acts selectively on subjects with a dysregulated $tPLA_2A$.

Keywords: Blood platelets, cognitive stimulation, mild cognitive impairment, phospholipases A2

INTRODUCTION

Phospholipases A_2 (PLA_2) form a superfamily of enzymes that catalyze production of lyso-phospholipids and free fatty acids by the hydrolysis of phospholipids sn-2 ester bond. They play a pivotal role in many physiological processes, including membrane remodeling and cell signaling [1, 2], and are involved in neurodegenerative disorders [3, 4].

PLA_2 modulation is a potential therapeutic target [5, 6]; in this context, cognitive stimulation (CS) is particularly promising, not only because in animal models it has effective regulating properties [7], but also because it is non-invasive, has no side effects, and presents no contraindications.

To date, only one study has been performed in humans: in a little cohort of healthy elderly subjects, a memory training intervention was proved to modulate platelet PLA_2 activity [8]. The use of platelet PLA_2 as peripheral biomarker of the neuronal enzyme is convincing in light of the recent finding that *total* PLA_2 ($tPLA_2$) activity in thrombocytes may mirror

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the *total* activity in the brain [9]. Moreover, platelets are widely considered "circulating neurons" because of the similarities existing between the two cells in terms of enzymes, receptors, and metabolic products [10, 11].

On these grounds, we evaluated the effects of CS on platelet $tPLA_2$ activity in a cohort of subjects with mild cognitive impairment (MCI).

MATERIALS AND METHODS

Subjects

All the subjects (74 healthy and 70 MCI) were enrolled at the Geriatrics Operative Unit of INRCA (Italian National Research Centres on Aging) in Fermo (Italy). The research was approved by the Institutional Ethical Committee (code SC/12/301) and each participant provided informed consent to participate to the study.

All subjects underwent a complete medical, neuropsychological, and functional evaluation; moreover, several laboratorial parameters (such as thyroid hormones, vitamin B12, and folic acid) as well as neuroimaging analyses (PET, CT, or MRI) were assessed to exclude any alterations that can determine cognitive deficits. MCI was diagnosed according to the criteria of Petersen et al. [12]. Patients under benzodiazepines, antidepressants, lipid lowering medications, non-steroidal anti-inflammatory drugs, anticoagulants, antihypertensive, and corticosteroids were included, and possible influence on platelet $tPLA_2$ activity was specifically evaluated.

The main characteristics of the populations are summarized in Supplementary Table 1A; inclusion and exclusion criteria are as in Casoli et al. [13].

Cognitive training

Each MCI subject was randomly assigned to either a multi-component cognitive training exercise group (EG; $n = 37$) or a control group (CG; $n = 33$) whose members received only some suggestions to improve specific outcomes. The protocol of cognitive training was applied as described [13]. Performances in digits span forward (auditory verbal short term memory [14]), Corsi supraspan (visuospatial short term memory [14]), attentive matrices (selective attention [15]), phonemic verbal and semantic fluency (linguistic abilities [16]), immediate and delayed prose recall (prose memory [15]), as well as word pairs learning (learning [16]) tests were used as outcomes.

Platelets isolation

Thirty milliliters of lithium heparin whole blood were drawn from each subject before cognitive training (baseline) and after termination (follow up (FU)). All drawings were done between 8:00 and 9:00 AM, in fasting state. Platelets were separated according to Rosenberg et al. [17], sonicated on ice (30 s at 8 μm of amplitude) and centrifuged at 10,000 × g for 15 min at 4°C. Supernatant aliquots were immediately stored at –80°C and tested within a month. Protein concentration was determined by the Lowry method [18]. Where not differently specified, all procedures were performed at room temperature.

$tPLA_2$ activity determination

Enzymatic activity was determined by a commercial kit ($cPLA_2$ Assay Kit, Cayman Chemical Company, Michigan, USA), normalized by protein concentration and expressed as nmol/min/mg. Since samples were not preliminarily purified for $sPLA_2$ or treated by $iPLA_2$-specific inhibitors, the data obtained can be referred to $tPLA_2$. All samples were measured in duplicates.

Statistical analyses

Results were expressed as means ± standard error of the mean (SEM) (continuous variables) or as percentage (categorical variables). Statistical comparisons were performed by t-Student test or by χ^2 test to compare the two groups at baseline; by paired t-Student test to evaluate the differences before and after the CS period; and by Pearson's coefficient to assess correlation between variables. The significance was accepted for $p < 0.05$.

RESULTS

The compliance to the study was 93.5% in CG and 87.9% in EG. "Not Evaluated" subjects at FU included those who did not complete the cognitive training/were not cognitively retested (dropout) or did not allow the drawn at the FU step; the overall dropout rate was 2.9%. The analyses performed at baseline included all the individuals enrolled.

Cognitive outcomes

Table 1 summarizes the cognitive outcomes of MCI patients evaluated at baseline and FU. In these

Table 1
Cognitive outcomes of control (CG) and trained (EG) MCI patients analyzed at baseline and FU

		Baseline score	FU score	*p*
Digits forward Test	CG	4.56 ± 0.130	4.47 ± 0.136	0.500
	EG	4.36 ± 0.151	4.65 ± 0.169	0.103
Corsi supraspan Test	CG	5.12 ± 0.125	5.01 ± 0.134	0.476
	EG	4.84 ± 0.165	5.17 ± 0.156	0.075
Attentive Matrices Test	CG	41.32 ± 1.574	40.04 ± 1.433	0.146
	EG	39.65 ± 1.661	43.39 ± 1.603	**0.010**
Semantic verbal fluency Test	CG	2.42 ± 0.240	2.40 ± 0.229	0.702
	EG	1.84 ± 0.239	2.13 ± 0.283	0.182
Phonemic Verbal fluency Test	CG	24.83 ± 1.365	24.01 ± 1.033	0.404
	EG	28.46 ± 1.315	31.27 ± 1.601	**0.018**
Immediate prose recall Test	CG	3.25 ± 0.398	3.09 ± 0.398	0.755
	EG	3.26 ± 0.383	4.23 ± 0.354	**0.037**
Delayed prose recall Test	CG	3.55 ± 0.499	2.81 ± 0.453	0.119
	EG	2.98 ± 0.448	4.11 ± 0.443	**0.038**
Total prose recall Test	CG	7.55 ± 0.761	6.71 ± 0.668	0.241
	EG	6.83 ± 0.683	8.80 ± 0.666	**0.006**
Word pairing Test	CG	6.91 ± 0.536	7.04 ± 0.501	0.802
	EG	8.65 ± 0.723	8.98 ± 0.824	0.517

In none of the tests, CG and EG showed statistical differences at baseline. The significant *p* values are marked in bold.

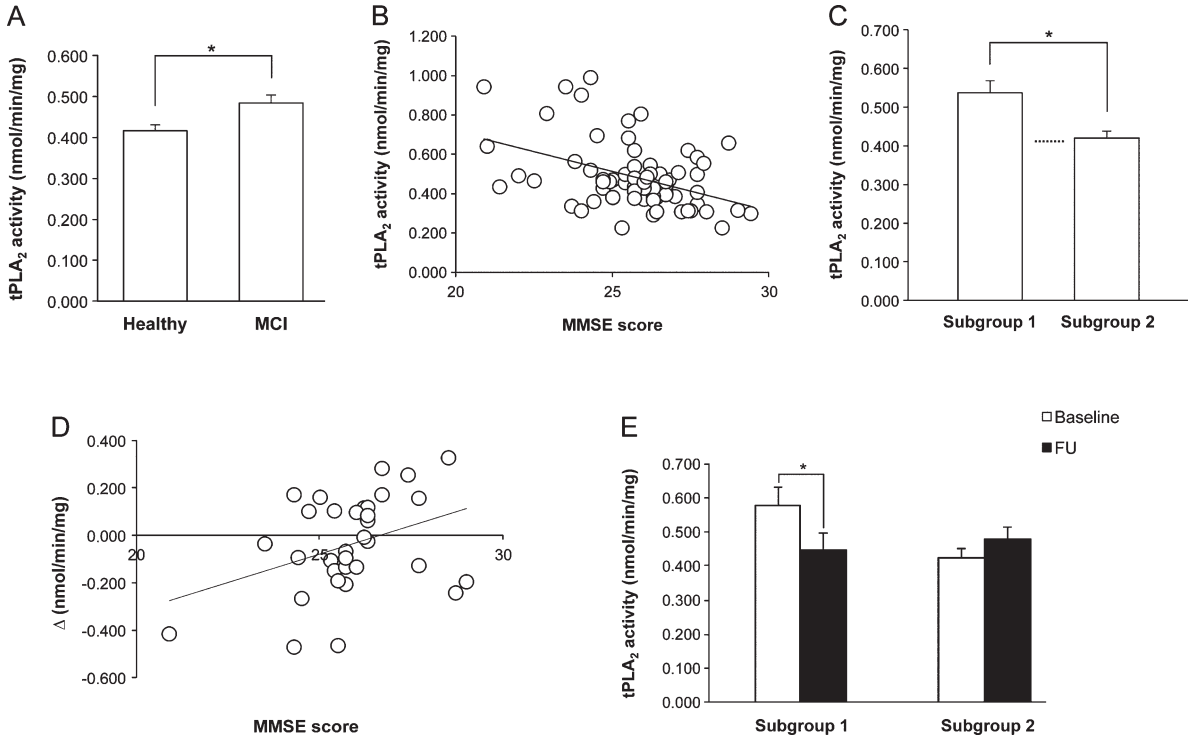

Fig. 1. A). Platelet $tPLA_2$ activity at baseline in healthy elderly and MCI subjects, who showed a significantly higher value. B) Correlation between MMSE score and $tPLA_2$ activity in MCI patients at baseline. Note that when MMSE values are higher, enzymatic activity is lower. C) $tPLA_2$ activity of MCI patients at baseline falls into two groups on the bases of the MMSE score: subjects with MMSE score <26 (Subgroup 1) had enzymatic activity significantly higher than subjects with MMSE score ≥26 (Subgroup 2), whose values were comparable to those of healthy elderly (dotted line). D) Correlation between the MMSE score at baseline and $tPLA_2$ activity changes before and after the intervention (Δ), in the experimental group of MCI patients. Δ = $tPLA_2$ activity at FU- $tPLA_2$ activity at baseline. E) Effect of cognitive stimulation on $tPLA_2$ activity of MCI patients Subgroups 1 and 2. Only in Subgroup 1, the training induced a significant decrease.

preliminary results, EG evidenced significantly increased performances at FU in attentive matrices, phonemic verbal fluency as well as immediate, delayed, and total prose recall tests. No significant differences were envisaged comparing baseline and FU in CG.

Platelet $tPLA_2$ activity at baseline

At baseline, $tPLA_2$ activity of MCI patients was significantly higher ($p = 0.008$) than that of healthy subjects (Fig. 1A), and the significance was maintained also when the data were adjusted for age and schooling by multiple linear regression analysis.

In the MCI group, a significant negative correlation was envisaged between the Mini-Mental State Examination (MMSE) score and the $tPLA_2$ activity ($R = -0.425$, $p < 0.001$) (Fig. 1B). The significance did remain also when the cohort was stratified for potentially confounding variables (i.e., gender, marital status, schooling, and age of pathology onset). To further analyze the correlation, the MCI group was divided according to the MMSE value, using the median as cut-off point: subjects with a score <26 (Subgroup 1, $n = 38$) had significantly higher $tPLA_2$ activity ($p = 0.003$) than individuals with a score ≥ 26 (Subgroup 2, $n = 32$), who showed values similar to the healthy elderly (Fig. 1C). The main characteristics of the two Subgroups are summarized in Supplementary Table 1B.

Effect of CS on platelet $tPLA_2$ activity

No significant differences were found between enzymatic activity at baseline and FU in controls (0.479 ± 0.0293 versus 0.499 ± 0.0445) or in experimental individuals (0.502 ± 0.0341 versus 0.476 ± 0.0277). However, in EG, a significant positive correlation was observed between $tPLA_2$ activity changes before and after the intervention (Δ is positive when the activity increases and negative when it decreases) and the MMSE score at baseline ($R = 0.366$, $p = 0.049$) (Fig. 1D); no significant correlation was found in controls ($R = -0.078$, $p = 0.675$), indicating that this phenomenon is training-specific. Thus, analyzing the CS effect in the two subgroups identified on the bases of the MMSE score at baseline, $tPLA_2$ activity showed a significant decrease in Subgroup 1 ($p = 0.019$), and no significant differences in Subgroup 2 at FU (Fig. 1E).

Drug influence

Drug use did not influence $tPLA_2$ activity, with the exception of antidepressants in the MCI group: patients ($n = 11$) who used these drugs had significantly lower values at baseline in comparison to untreated MCI subjects (0.417 ± 0.0255 versus 0.496 ± 0.0231, $p = 0.028$). Excluding these 11 subjects, the significant differences and correlations remained unchanged.

DISCUSSION

The present study showed that in subjects with MCI, platelet $tPLA_2$ activity correlates with patients' cognitive conditions, and that CS acts selectively on the enzyme, i.e., it modulates the parameter only in individuals with deregulated values in comparison to the healthy elderly.

Based on the MMSE score, it was possible to subdivide at baseline the MCI cohort into two subgroups: patients with more evident cognitive impairment (MMSE score <26) and significantly higher $tPLA_2$ activity, and individuals cognitively more preserved (MMSE score ≥ 26), who had $tPLA_2$ activity similar to the healthy elderly. The finding that the increase of $tPLA_2$ activity and the severity of the global cognitive status impairment are significantly linked suggests a possible role of $tPLA_2$ in MCI progression. PLA_2 activity alterations may lead to the synthesis of excessive proinflammatory mediators and peroxidative products [19], and inflammation and oxidative stress may contribute to the pathogenesis of Alzheimer's disease (AD) [20, 21], of which MCI could be a prodromal condition. It is therefore conceivable that the more deregulated $tPLA_2$ is, the more harmful molecules might be released, and the more severe the pathological consequences might become. The finding that in patients affected by AD $tPLA_2$ activity is significantly higher than in healthy controls [22, 23] as well as in MCI subjects [23] is in line with this hypothesis.

As far as the therapeutic potentialities of CS are concerned, the protocol not only exerted positive effects on several cognitive outcomes, but also counteracted the peripheral enzymatic deregulation. Indeed, CS improved parameters linked to memory, attention, and verbal, confirming the results of others [24]. It is worth noting that CS acts on $tPLA_2$ activity in a "dysfunction-dependent" mode: in subjects with an initial enzymatic activity higher than in the healthy elderly (Subgroup 1), CS reduced the

value; in subjects with an initial enzymatic activity similar to the healthy elderly (Subgroup 2) CS did not induce any significant change. Thus, CS seems to have homeostatic properties on $tPLA_2$ activity. This result may seem in contradiction with the observation that in the healthy elderly CS induces platelet $tPLA_2$ increase [8]. Actually, it is conceivable that, in absence of pathology, increased activity produced by the training improves cell functioning while in MCI, where the increased values might be linked to inflammation and oxidative stress, the protocol acts in the opposite way. Indeed, recent evidence supports the use of specific PLA_2 inhibitors as preventive/therapeutic agents for inflammatory disorders [25], and several studies showed that environmental enrichment exert anti-inflammatory and neuromodulatory effects [26]. Thus, in MCI and AD, where the involvement of neuroinflammation is well established [27, 28], CS may produce a down regulation effect in the central nervous system, which might influence also circulation blood components.

In conclusion, this study suggests that platelet $tPLA_2$ activity may be useful as peripheral biomarker to differentiate MCI patients at different pathological stages, and sustains the use of CS as non-pharmacological therapeutic strategy.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150714.

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