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# Can fibrinolytic system components explain cognitive impairment in multiple sclerosis?

María Yaiza Pérez-Martín[a,*], Montserrat González-Platas[a], Alejandro Jiménez-Sosa[b], Julio Plata-Bello[c,d], Francisco Javier Carrillo-Padilla[a], Andrés Franco-Maside[e], Pablo Eguia-del Río[f]

[a] Department of Neurology, Complejo Hospitalario Universitario de Canarias, Santa Cruz de Tenerife, Spain
[b] Unit of Research, Complejo Hospitalario Universitario de Canarias, Santa Cruz de Tenerife, Spain
[c] Department of Physiology, Faculty of Medicine, University of La Laguna, San Cristóbal de La Laguna, Spain
[d] Department of Neurosurgery, Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain
[e] Department of Immunology, Complejo Hospitalario Universitario de Canarias, Santa Cruz de Tenerife, Spain
[f] Department of Neurology, Hospital Dr. José Molina Orosa, Ctra. Arrecife. Lanzarote. Las Palmas de Gran Canaria, Spain

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ABSTRACT

*Background:* The fibrinolytic system is capable of modulating inflammatory and degenerative events within the central nervous system. Specifically, the plasminogen activator inhibitor-1 (PAI-1) has been associated with different pathological conditions in multiple sclerosis (MS) and its role in cognitive functioning is also known.
*Objectives and methods:* To study the association between plasma levels and the polymorphic variants of the PAI-1 gene and cognitive performance in MS. 176 patients were studied. Neuropsychological evaluation was performed with the Brief Repeatable Battery of Neuropsychological Tests (BRB-N). A Polymerase Chain Reaction (PCR) was used to determine PAI-1 4G/5G polymorphisms and quantification was performed using an Enzyme-Linked ImmunoSorbent Assay (ELISA).
*Results:* Participants were categorized as not cognitively impaired (NCI; n = 114) and cognitively impaired (CI; n = 62). The NCI group had a higher percentage of heterozygous subjects but no statistical differences were found between the CI and NCI group. Neuropsychological functioning did not correlate with plasma levels of PAI-1 or its genetic polymorphism. It is noteworthy that PAI-1 plasma levels were related to neurological impairment.
*Discussion:* Cognitive impairment in MS is due to strategic focal lesions affecting regions and tracts involved in cognitive processes and to diffuse damage in the white and gray matter. This complex etiology could explain the absence of a relationship between the cognitive functioning and PAI-1 in patients with MS that has been found in vascular dementia or Alzheimer's disease. Plasma curves of PAI-1 and its measures in cerebrospinal fluid could help elucidate the role of PAI-1 in MS.

## 1. Introduction

Interactions between cells are a fundamental part of multicellular entities and help to maintain the balance and good functioning of the organism. These interactions involve a large number of molecules that have different functions according to the type of tissue they form and their location.

A tissue is mainly composed of cells, but also of the extracellular matrix (ECM), which serves as a support to and conformation of the tissue itself. The ECM is a dynamic structure in a constant state of remodeling that is composed of a large variety of molecules, and an amorphous fundamental structure in the form of a gel.

In the human brain, the ECM is a complex network of proteins and proteoglycans that regulates synapses and maintains their stability, which is why some authors consider it to be a structural support that surrounds neurons and glia, including the adjacent space and the space between the synapses [1].

Among the functions proposed for ECM molecules in the brain are: formation and stabilization of synapses, maintenance of ionic homeostasis, modulation of certain neurotransmitters, participation in developmental phenomena, differentiation and migration of glial cells, among others [2,3].

* Corresponding author at: Service of Neurology, Hospital Universitario de Canarias, Ofra, s/n. La Laguna, 38320 Santa Cruz de Tenerife, Spain.
*E-mail address:* yaizaneuro@hotmail.com (M.Y. Pérez-Martín).

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The constant remodeling of ECM depends on different elements including the fibrinolytic system (FS) or the plasminogen system. The FS is a natural defense mechanism of the organism responsible for the elimination of clots. Its activation is carried out by the coordinated action of activators and inhibitors, thereby maintaining a dynamic balance with the coagulation system to ensure vascular permeability [4].

The FS is composed of molecules that, in the last instance, convert plasminogen into its active form, plasmin, which is able to degrade fibrin and activate the metalloproteinases (MMP) of the ECM [5] which play an important role in tissue remodeling and in mechanisms involved in cell migration and invasive growth [6].

The physiological activators of plasminogen are activated by factors XII and XI, urokinase (u-PA) and the tissue plasminogen activator (t-PA). u-PA plays an essential role in neural cell migration, axonal growth and branching [7]. While t-PA is a single-stranded serine protein, synthesized by endothelial cells and released into circulation by stimuli such as stress, physical exercise, venous occlusion or administration of vasoactive drugs. t-PA promotes thrombolysis, restitution of blood flow and possibilities of brain tissue recovery. The rupture of the blood-brain barrier (BBB) of the ischemic zone allows the passage of plasma t-PA in high concentrations to the central nervous system (CNS), where it has a detrimental effect due to the possible activation of certain MMP which enhance the damage in the adjoining areas.

The main inhibitor of the plasminogen activator is PAI-1 (plasminogen activator inhibitor-1). PAI-1 blocks the conversion of plasminogen to plasmin. Platelets make up the highest concentration of PAI-1 in the blood compartment. The PAI-1 gene is located in the q21.3-q22 region of chromosome 7 [8] and its different polymorphisms increase or decrease plasma concentrations of PAI-1, which may favor or hinder the development of cerebrovascular disease, cognitive deterioration or inflammatory disease [5]. Homozygous subjects of the 4G allele have higher levels of PAI-1 than homozygous subjects of the 5G allele due to interaction with a transcriptional repressor protein, which has a higher affinity for the 5G allele [9].

Plasma concentrations of PAI-1 are very low, but higher than those of activators and are characterized by fluctuations in relation to the time of day, or caffeine and carbohydrate intake and increase in relation to insulin, adrenaline, angiotensin II, administration of corticosteroids and increase of certain cytokines [10]. Plasma concentrations of active PAI-1 vary in healthy subjects between 0 and 40 U/mL, whereas those in PAI-1antigen range from 5 to 100 ng/mL [11].

### 1.1. Fibrinolytic system and multiple sclerosis

In humans, the BBB regulates the entry of proteins and other components present in the bloodstream into the cerebral parenchyma. Recent research on the potential role of fibrinolysis in the pathogenesis of multiple sclerosis (MS) suggests that inflammation, focal demyelination and axonal degeneration occur following BBB disruption and entry into the CNS of serum proteins, including fibrinogen [12] that are not physiologically present in nerve tissue [13] and are capable of altering the composition of the ECM [14].

The FS can modulate inflammatory and degenerative events in the CNS, and its manipulation can have therapeutic effects in diseases such as multiple sclerosis [13].

t-PA is able to intervene in the process of demyelination in experimental acute encephalomyelitis (EAE), an inflammatory disease of the CNS, mediated by CD4 + T cells which share histopathology with MS. [15] t-PA may increase the permeability of the BBB, which facilitates the entry of inflammatory cells into the CNS, and intervenes in the cascade of inflammatory events culminating in the clinical manifestations of the disease [16]. t-PA also contributes to neuronal degeneration in the early stages of EAE and its absence causes a delay in the onset of the disease, although in later stages the presence of t-PA may be beneficial for neuronal regeneration [17].

The over-regulation of PAI-1 contributes to the deposition of fibrin, which hinders axonal regeneration. Elimination of fibrin in EAE suppressed the development of the disease and reduced the neurological deficit [13].

Cerebrospinal fluid (CSF) studies have demonstrated significantly higher t-PA concentrations (up to 10-fold higher) in patients with MS than in controls. In addition, increased t-PA activity has been linked to disease progression [18].

Inflammatory activity in MS has been associated with plasma levels of PAI-1 Thus, plasma levels of PAI-1 in participants with active MS during the first 14 days of relapse (who had relapse during the 6-month observation period) were 6 times higher than those in the control group and were significantly higher than participants with stable MS (free of relapses during the study) [19]. There were no significant differences in plasma levels of PAI-1 between stable participants and the control group. Interestingly, the same study, reported that, 14 days after relapse, PAI-1 levels returned to normality suggesting this is related to the brevity of the action of PAI-1 as a homeostatic response to inflammation.

Significant concentrations of u-PA, u-PA receptor and PAI-1 have been found in acute MS plaques and u-PA receptors in normal-appearing white matter [20]. The three proteins were localized in perivascular regions which could facilitate cellular infiltration into the CNS.

Some studies have shown that increased PAI-1 reduces fibrinolytic activity in MS lesions and therefore contributes to the elimination of fibrin and axonal damage. However, small amounts of active t-PA may continue to be protected between macrophages and play a role in fibrin dissolution [21]. It appears that the limited availability of t-PA in MS lesions due to the formation of PAI-1 reduces the ability of t-PA receptors to generate plasmin, which further decreases fibrinolytic capacity in active MS lesions and possibly protects against axonal damage. These vascular changes may even precede the formation of lesions [22,23].

Genetic studies, show that PAI-1 genotype 5G/5G appears to be a low-level producer of PAI-1, implying a risk factor for MS in women [24] whereas the 4G/4G genotype appears to be a protective factor [25].

On a therapeutic level, none of the PAI-1 antagonists have been investigated in the CNS in disease models because of their inability to cross the BBB. In a recent study, effects of TM5484 (after optimizing and increasing its penetrating properties) and methylprednisolone and fingolimod (as relapse and as disease modifying, respectively) were compared [26]. TM5484 showed therapeutic effects and neuroprotector capacities.

### 1.2. Cognition and fibrinolytic system

Cognitive impairment is a common manifestation of a variety of neurological disorders. This feature affects up to 65% of patients with MS [27] at both the earlier and later stages of the disease [28], and it tends to worsen over time [29]. MS negatively affects several aspects of cognitive functions, including attention, information processing [30], learning and memory, executive function and visuospatial abilities [31], having an important impact on quality of life [32–34], employment status, [35] daily functioning, independence [36] and participation in social activities [37]. The pathogenesis of cognitive impairment in MS is still not completely clear.

The role of some of the components of FS in cognitive functioning has been studied in both mice and humans.

There are data from the performance of the t-PA/plasmin system as a modulator of NMDA receptors (*N*-methyl-D-aspartate). NMDA receptors are ionotropic glutamate receptors, NMDA is the main excitatory neurotransmitter involved in brain functioning, whose concentrations in the thalamus, hippocampus and the cingulate region are involved in the visuospatial memory in MS patients [38] facilitating signaling of the NMDA receptor, reducing stress-induced anxiety [39]