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Increased tissue factor pathway inhibitor and homocysteine in Alzheimer's disease

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Abstract

We investigated the possible involvement of vascular damage in the pathogenesis of Alzheimer's disease (AD), by assessment of plasma levels of tissue factor pathway inhibitor (TFPI), a serine protease inhibitor induced by endothelial injury, and homocysteine (Hcy), a known risk factor for cerebrovascular disorders, folate levels were also measured. 110 probable AD, 38 mild cognitive impairment, 31 patients affected by idiopathic Parkinson's disease (without dementia) and 100 healthy controls, who displayed no vascular disorders were enrolled. TFPI and Hcy were significantly higher in AD patients with respect to other groups. The levels of TFPI and Hcy were positively correlated in hyperhomocysteinemic AD and mild cognitive impairment subjects, and were negatively correlated with folate levels. Our findings suggest that an impairment of endothelial function associated with high Hcy levels may occur in AD patients, despite the absence of manifest cerebrovascular lesions. Therefore, TFPI may represent a candidate marker of endothelial damage in AD and might be used for the identification and monitoring of patients that would benefit from folate supplementation treatment.

Keywords: Alzheimer's disease; Endothelial injury; Homocysteine; Tissue factor pathway inhibitor

1. Introduction

Although Alzheimer's disease (AD) is mainly considered a neurodegenerative disorder, much evidence points to the additional involvement of cerebrovascular pathology. Besides degenerative neuropathological markers, i.e., neuronal loss, neuritic plaques, and neurofibrillary tangles, the AD brain also shows significant structural damage of blood vessels' wall: atherosclerosis, fibrosis, inflammatory changes, and beta-amyloid (Abeta) protein deposits (de La Torre, 2000; Hamel et al., 2008). Moreover, most risk factors for cerebrovascular disorders are also related to Alzheimer's disease (van Oijen et al., 2007).

Cerebrovascular pathology is known to contribute to dementia by causing silent brain infarcts (SBI), which have an additive effect with respect to neurodegeneration in determining cognitive impairment (Jellinger, 2007). Furthermore, vascular factors triggered by Abeta deposition and hypoxia may contribute to neuronal damage in AD, putatively via phenomena taking place in the endothelium of cerebral vessels. Endothelial dysfunction has been demonstrated in AD patients (Iadecola, 2004; Zlokovic, 2005) and may also precede neurodegenerative changes (de La Torre, 2005; Shi et al., 2000). Blood brain barrier (BBB) and cortical cerebral blood flow (CBF) changes would in turn

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increase vascular permeability, causing the passage into the perivascular neural parenchyma of serum molecules such as homocysteine (Hcy) and coagulation mediators, both able to induce oxidative stress (Kamath et al., 2006; Tomimoto et al., 1996).

Thus, the cerebral vessels' endothelium might represent the critical locus of interaction between degenerative and vascular pathogenetic factors in the AD brain. Accordingly, biochemical parameters related to the endothelial damage might be useful to investigate the involvement of vascular factors in AD.

The tissue factor pathway inhibitor (TFPI) is the key player in the extrinsic coagulation cascade and is the only known regulator of the tissue factor (TF)-dependent pathway (Lwaleed and Bass, 2006). TFPI is a Kunitz-type serine protease inhibitor constitutively produced by endothelial cells, plasma, platelets, and macrophages (Novotny et al., 1989; Sandset, 1996), which binds and inhibits TF-activated factor VII (FVIIa) in a FXa-dependent manner (Ott et al., 2001). The brain is very rich in TF and changes in TF levels have been demonstrated in the early phase of the acute stroke, inducing an upregulation and release of TFPI from disrupted endothelium and activated platelets (Adams et al., 2006). Moreover, increased plasma levels of TFPI, evidence of chronic cerebral endothelial dysfunction and prothrombotic alterations, have been demonstrated in patients with cerebral small vessel disease (SVD) (Hassan et al., 2003). Interestingly, TFPI has also been immunohistochemically localized in senile plaques in both AD and non-AD subjects, while increased TF and TFPI levels have been found only in the frontal cortex of AD patients (Hollister et al., 1996; McComb et al., 1991).

Elevated plasma levels of Hcy, a condition called hyperhomocysteinemia (HHcy), is one of the strongest independent risk factors for vascular and cerebrovascular disorders (Bostom et al., 1999; Perry et al., 1995; Selhub et al., 1995) and HHcy has been demonstrated to be able to induce oxidative stress in human platelets, leading to the increased formation of thromboxane B_2 (TxB_2) as the inactive product of the potent platelet-aggregating agent thromboxane A_2 (Signorello et al., 2002).

Brain microcirculation may be particularly susceptible to the endothelial dysfunction caused by Hcy (Kamath et al., 2006). Recent studies have demonstrated that HHcy is associated with an increased risk of developing AD (Seshadri et al., 2002), with a reduced brain volume and with the presence of SBI in healthy adults (Das et al., 2008; Seshadri et al., 2008).

Despite numerous data which support the neurovascular hypothesis in AD pathogenesis (Zlokovic, 2005), there is no clear in vivo evidence about a direct linkage between HHcy and the possible endothelial damage in AD patients.

We performed the current study to suggest TFPI as a useful biological marker related to the endothelial dysfunction in patients with mild cognitive impairment (MCI) and mild "pure" AD, to advance the notion that microvascular injury is associated with AD independently of significant signs of peripheral vascular or cerebrovascular disease. To reinforce our hypothesis that TFPI levels increase because of the presence of endothelial damage produced by HHcy, that could finally lead to an increased prothrombotic state, we also evaluated the TF and TxB_2 plasma levels, in a subgroups of AD patients.

Because plasma Hcy levels are readily modifiable by vitamin supplementation and since, very recently, it has been demonstrated to correlate with Abeta (Tangney et al., 2009), we also aimed to test a possible association between Hcy-induced endothelial dysfunction and both folic acid and vitamin B12, 2 of the most important cofactors involved in methionine metabolism (Leboeuf, 2003).

2. Methods

2.1. Subjects

Consecutive sampling was used to enroll AD and MCI subjects among patients attending the Neurology Units of S. Gerardo Hospital, University of Milano Bicocca, and Neurology Unit, University of Brescia, Italy. No case of familial AD was included. Only patients with "probable" AD were included; the diagnosis was made according to Diagnostic and Statistical Manual of Mental Disorders-IV (DMS-IV) and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984). Diagnostic criteria for amnesic MCI (aMCI) were those defined by Petersen et al. (1999). All patients underwent neurological examination, routine blood tests (including thyroid hormones, folic acid, vitamin B12, neurosyphilis serology), magnetic resonance imaging (MRI), and their cognitive and behavioral status assessed with a comprehensive standardized neuropsychological battery.

As a pathological non-AD neurodegenerative disorder control group we enrolled patients affected by idiopathic Parkinson's disease (without dementia), defined according to Gelb et al.'s (1999) criteria, while the healthy control group included caregivers and elderly volunteers with no sign of cognitive impairment at the Mini Mental State Examination (MMSE) cut-off value for inclusion ($\geq$26.0), and no exclusion criteria at a medical and pharmacological interview.

AD and Parkinson's disease with a history, physical signs, and/or imaging evidence of cerebrovascular disease were excluded. More precisely, AD and amnesic MCI patients were excluded if their brain magnetic resonance imaging scan indicated large or small vessel cerebrovascular disease, including prior hemorrhagic or ischemic lesions >1 cm^3 and multiple cortical or subcortical lacunae. Mild chronic ischemic white matter disease or scattered lacunar infarcts were accepted unless the patient's neurological history and cognitive profile suggested a vascular genesis.