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Menopausal transition: A possible risk factor for brain pathologic events

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Abstract

Background and objective: Incidence and prevalence of Alzheimer's disease (AD) are higher in postmenopausal women than in age-matched men. Since at menopause the endocrine system and other biological paradigms undergo substantial changes, we thought to be of interest studying whether (and how) the balance between some biological parameters allegedly neuroprotective (e.g. related to estrogen, dehydroepiandrosterone and CD36 functions) and others considered pro-neurotoxic (e.g. related to glucocorticoid and interleukin-6 activities) vary during lifespan in either sex in either normalcy or neurodegenerative disorders.

Subjects and methods: Along with this aim, we evaluated the gene expression levels of estrogen receptors (ERs), glucocorticoid receptors (HGRs), interleukin-6 (IL-6) and CD36, a scavenger receptor of class B allegedly playing a key role in the proinflammatory events associated with AD, in a population of 209 healthy subjects (73M, 106F, 20–91-year old) and 85 AD patients (36M, 49F, 65–89-year old). Results obtained were related to plasma titers of estrogens, cortisol and dehydroepiandrosterone sulfate (DHEAS). Studies were performed in peripheral leukocytes, since these cells (1) are easily obtainable by a simple blood sampling, (2) express many molecules and multiple receptors which are under the same regulatory mechanisms as those operative in the brain and (3) some of them, e.g. monocytes, share many functions with microglial cells.

Results: In healthy men all the study parameters were quite stable during lifespan. In women, instead, at menopausal transition, some changes that may predispose to neurodegeneration occurred. In particular, there was (1) an up-regulation of ERs, and a concomitant increase of IL-6 gene expression, events likely due to the loss of the inhibitory control exerted by estradiol (E_2); (2) an increase of HGRα:HGRβ ratio, indicative of an augmented cortisol activity on HGRα not sufficiently counteracted by the inhibitory HGRβ function; (3) a reduced CD36 expression, directly related to the increased cortisol activity; and (4) an augmented plasma cortisol:DHEAS ratio, widely recognized as an unfavorable prognostic index for the risk of neurodegeneration. In AD patients of both sexes, the expression of the study parameters was similar to that found in sex- and age-matched healthy subjects, thus indicating their unrelatedness to the disease, and rather a better correlation with biological events.

Conclusions: Menopausal transition is a critical phase of women's life where the occurrence of an unfavorable biological *milieu* would predispose to an increased risk of neurodegeneration. Collectively, the higher prevalence of AD in the female population would depend, at least in part, on the presence of favoring biological risk factors, whose contribution to the development of the disease occurs only in the presence of possible age-dependent triggers, such as beta-amyloid deposition.

Keywords: Menopausal transition; Hormone receptors; CD36; Leukocytes; Alzheimers disease; Estrogen receptors; Glucocorticoid receptors; IL-6

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1. Introduction

As the age distribution of the population shifts toward an increase, the dementing disorders, especially Alzheimer's disease (AD), are emerging as a major worldwide health problem. To ameliorate the comprehension of the pathogenetic events underlying neurodegeneration, many prevalence studies on dementia and AD have been conducted in various population subgroups. In particular, the effects of gender have been investigated. Although conflicting data have been reported (Brayne et al., 1995; Nilsson, 1984), most of the studies support a higher prevalence and incidence of AD in women, even after adjusting for their differential survival (Bachman et al., 1992, 1993; Gao et al., 1998). This has obviously focused the attention on the role of female hormones, e.g. estrogens, whose production dramatically decreases at menopause.

The role of estrogens in AD has been investigated in a variety of *in vivo* and *in vitro* models. In these studies, estrogens have been shown to be potent neuroprotective agents. In fact, they (a) augment the cerebral blood flow in the hippocampus and temporal lobe, two brain areas involved in the early pathological changes of AD (Maki and Resnick, 2000, 2001); (b) exert neurotrophic actions on different neuronal populations (Gibbs and Aggarwal, 1998; Granholm et al., 2002, 2003; Leranth et al., 2000; McEwen, 2002); (c) decrease cholesterol levels and modulate the expression of the gene encoding apolipoprotein E (ApoE) (Brinton et al., 2000; Lambert et al., 2004); (d) prevent the formation of beta-amyloid (βA) fibrils and protect the cells against their cytotoxic effects (Granholm et al., 2003; Thomas and Rhodin, 2000); (e) inhibit the chronic inflammatory reaction that has a pathogenetic role in AD (Thomas and Rhodin, 2000); (f) induce the synthesis of thioredoxin, a multifunctional protein endowed with antioxidant and neuroprotective actions (Chiueh et al., 2003). Inferential support to the protective role of estrogens in AD rests on the observation that cognitive function is improved by hormone therapy (HT) in postmenopausal women (Jacobs et al., 1998; Phillips and Sherwin, 1992).

Despite this large body of evidence, other studies have denied the alleged protective role of estrogens, leaving the problem unsettled (den Heijer et al., 2003; Espeland et al., 2004; Shumaker et al., 2003, 2003). Moreover, the Women's Health Initiative Memory Study (WHIMS), a wide randomized placebo-controlled clinical trial for HT in postmenopausal women, has recently shown that in women with an average age of 63 years at entry, HT increases the risk of probable dementia (Shumaker et al., 2003, 2004), and hypothesized that the negative effect may be related to the HT-induced increased risk of stroke, standing the strong relationship existing between microinfarcts in the brain and susceptibility to AD (Shumaker et al., 2003, 2004). For a thorough discussion, see Turgeon et al. (2006).

With these disparate findings in mind, different authors have hypothesized the existence of a "critical temporal window", likely coincident with the menopausal transition, within which the estrogens manifest their positive effects and over which, instead, they become detrimental (Kesslak, 2002; Smith and Levin-Allerhand, 2003; Zandi et al., 2002). Along this line, it is noteworthy that in postmenopausal women the reduction of the risk of dementia is related to the previous and not to the current use of estrogens (Zandi et al., 2002).

Among elderly, and particularly in AD patients, a disrupted hypothalamo-pituitary-adrenal function may also play a role in neurodegeneration (Murialdo et al., 2001). Higher glucocorticoid levels, in fact, may alter the function of hippocampal neurons and glial cells, rendering these elements more vulnerable to metabolic insults, such as hypoglycaemia and hypoxia. They also cause synaptic disruption and are involved in neuronal cell death (Müller, 2001; Sapolsky et al., 1991).

In the last decade, search for biological and hormonal markers of dementia expressed in easily accessible tissues has been intensified. This led to identify several molecules, whose diagnostic potential is now under investigation. Among them, particularly promising would be CD36, a multifunction protein belonging to the family of the class B scavenger receptors. CD36 is expressed on microglia of normal and AD brains and would play an important role in the proinflammatory events associated with AD (Christie et al., 1996; Coraci et al., 2002; El Khoury et al., 1996; Husemann et al., 2001; Maxeiner et al., 1998). Recently, we have shown that CD36 is also expressed by peripheral leukocytes and that its expression by these cells is lower in AD patients than in age-matched controls (Giunta et al., 2006).

Peripheral leukocytes express virtually all hormones and hormone receptors, which are under the same regulatory mechanisms that control their expression in the brain (Hori et al., 1991; Kim and de Vellis, 2005). Importantly, the prevailing view about origin of microglia is that it derives from peripheral leukocytes, particularly, monocytes which, during embryonic development, enter the brain from the bloodstream and then differentiate into brain resident microglia, displaying several cell surface antigens described in monocytes (Kim and de Vellis, 2005). Hence, these cells, easily obtainable *via* a simple blood sampling, may be profitably used as tools to investigate the changes occurring in brain areas reportedly inaccessible in humans.

These premises, dictated the study of the leukocyte expression of some biological parameters in a large group of normal non-dementing subjects and AD patients of either sex, the aim being that of evaluating how the balance between neuroprotective/neurotoxic influences varies across life. Our attention focused on the expression of estrogen and glucocorticoid receptors and the production of interleukin-6 (IL-6), a proinflammatory molecule likely involved in the pathogenesis of AD (Papassotiropoulos et al., 2001). Results were compared to the leukocyte expression of CD36 and related to the circulating levels of estrogens, cortisol, and dehydroepiandrosterone sulfate (DHEAS).