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Proteomic analysis of peripheral leukocytes in Alzheimer's disease patients treated with divalproex sodium

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Abstract

The molecular profiling of peripheral tissues, including circulating leukocytes, may hold promise in the discovery of biomarkers for diagnosing and treating neurodegenerative diseases, including Alzheimer's disease (AD). As a proof-of-concept, we performed a proteomics study on peripheral leukocytes from patients with AD both before and during treatment with divalproex sodium. Using two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry, we identified 10 differentially expressed proteins: two up-regulated proteins, 14-3-3 protein ε and peroxiredoxin 2; and eight down-regulated proteins, actin-interacting protein, mitogen activated protein kinase 1, beta actin, annexin A1, glyceraldehyde 3-phosphate dehydrogenase, transforming protein RhoA, acidic leucine-rich nuclear phosphoprotein 32 family member B, and a currently unidentified protein. A subset was validated on both the transcript and protein levels in normal human peripheral blood mononuclear cell cultures treated with valproic acid. These proteins comprise a number of functional classes that may be important to the biology of AD and to the therapeutic action of valproate. These data also suggest the potential of using peripheral leukocytes to monitor pharmaceutical action for neurodegenerative diseases.

Keywords: 2D electrophoresis; Alzheimer's disease; Biomarkers; Glycogen synthase kinase-3; GSK-3; Histone deacetylase inhibitor; HDAC; PBMC; Proteomics; qRT-PCR; Valproic acid; VPA

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that results in the death of specific neuronal populations in a temporally and spatially distinct pattern and subsequent cognitive, functional, and behavioral impairments. Current FDA approved treatment options for AD are limited to two classes of drugs, those that enhance cholinergic function via inhibition of acetylcholinesterase and those that alter glutamatergic synaptic function via uncompetitive,

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low-to-moderate affinity NMDA-receptor antagonism. Neither drug class has been shown to alter disease progression. However, disease-modifying treatments are in preclinical or clinical development that target a variety of AD-related pathologies, including amyloid beta (Aβ) plaques, hyperphosphorylated tau-containing neurofibrillary tangles (NFT), oxidative stress, and inflammation.

Other therapeutics are also widely prescribed to help alleviate the neuropsychiatric and behavioral features of AD, which are estimated to affect greater than 80% of patients with dementia (Lyketsos et al., 2002; Weiner et al., 2005) and often lead to a number of adverse outcomes, including increased caregiver burden, earlier institutionalization, and decreased quality of life. One such treatment is the short-chain branched fatty acid, valproic acid (2-propylpentanoic acid, valproate; VPA), which is often administered to patients as the divalproex sodium formulation. Some studies suggest that VPA may afford symptomatic relief of agitation in people with dementia (Porsteinsson et al., 1997, 2003; Sival et al., 2002; Tariot et al., 2002), although the most recent study found no benefit (Tariot et al., 2005). A multicenter clinical trial is currently being conducted by the Alzheimer's Disease Cooperative Study (ADCS) that is investigating the potential for VPA to attenuate progression of the clinical features of AD (Tariot et al., 2002).

While its mechanisms of action remain unclear, a number of studies suggest that VPA inhibits the activity of the histone deacetylases (HDACs) (Bradbury et al., 2005; Gottlicher et al., 2001; Gurvich et al., 2004; Phiel et al., 2001; Yildirim et al., 2003), which in turn leads to derepression of gene transcription. Other studies have also shown that VPA inhibits a major signaling enzyme, glycogen synthase kinase-3 beta (GSK3β; Chen et al., 1999; Kim et al., 2005), although this effect may be an indirect function of more global changes resulting from HDAC inhibition (De Sarno et al., 2002; reviewed in Gurvich and Klein, 2002). Regardless, VPA appears to exert complex effects on diverse molecular signaling pathways and a number of studies have demonstrated its neuroprotective potential (Dou et al., 2003; Hashimoto et al., 2002; Jeong et al., 2003; Mora et al., 1999; Morland et al., 2004; Wang et al., 2003). Thus, while additional research is necessary, these findings suggest the possibility that VPA and similar drugs could alter the course of AD (reviewed in Chuang, 2005; Loy and Tariot, 2002; Tariot et al., 2002).

In addition to alterations within the central nervous system (CNS) AD has both systemic manifestations and resultant compensatory responses that affect a number of peripheral tissues. This suggests the potential use of these tissues in understanding disease biology, progression, and therapeutic actions. Studies in fibroblasts from people with AD have found alterations in proteins related to the disease compared to controls (Mazzola and Sirover, 2001; Scali et al., 2002; Takeda et al., 1992; Zhao et al., 2002, 2003) and Nagasaka et al. (2005) recently reported a unique gene expression profile in people with one of three familial AD mutations (APP_{SWE}, APP_{ARC}, or *PSEN* H163Y) compared to their unaffected (wild-type) siblings. Other studies have found AD-specific changes in peripheral leukocytes that mirror changes in neurons, including alterations in glucose utilization (Blum-Degen et al., 1995; Urcelay et al., 2001), increased sensitivity to apoptotic stimuli (Blandini et al., 2006; Eckert et al., 1998; Mecocci et al., 2002; Morocz et al., 2002; Velez-Pardo et al., 2002), cell cycle re-entry (de las Cuevas et al., 2003; Marx et al., 1999; Nagy et al., 2002; Urcelay et al., 2001), increased oxidation (Cecchi et al., 1999; Kadioglu et al., 2004; Leutner et al., 2005; Straface et al., 2005), altered calcium signaling (de las Cuevas et al., 2003; Eckert et al., 1996; Mattson et al., 2001; Palotas et al., 2002), and a variety of specific proteomic (Hye et al., 2005; Jabbour et al., 1992; Jung et al., 1999; Mirinics et al., 2002; Tacconi et al., 2004; Tayebati et al., 2001) and transcriptomic changes (Coleman et al., Personal communication; Ebstein et al., 1996; Kalman et al., 2005). Peripheral leukocytes have also been used to study molecular changes in response to therapy in AD (Casademont et al., 2003; Gambi et al., 2004; Palotas et al., 2004a, 2004b; Reale et al., 2004, 2005; Wong et al., 2004). Finally, a recently published microarray study demonstrated the utility of biomolecular profiling of peripheral leukocytes in patients with Huntington's disease (HD) (Borovecki et al., 2005). A number of significantly elevated transcripts were identified in leukocytes from patients compared to control subjects, which were also found to be altered within the disease-affected caudate region in brains of HD patients and whose expression levels within leukocytes were abrogated following treatment with the HDAC inhibitor, sodium butyrate. Taken in aggregate, these studies illustrate a rationale for the use of peripheral tissues, including leukocytes, as reporters of central nervous system (CNS) diseases and as potential monitors of therapy.

We have developed a working hypothesis that VPA-induced alterations in genes and gene products in peripheral leukocytes are reflective of generalized molecular and cellular alterations and may provide important information regarding the effects of VPA on the CNS. As an initial proof-of-concept, we set out to determine differential protein expression profiles in peripheral leukocytes in AD patients on VPA therapy. Using both unbiased discovery as well as validation approaches, we demonstrate VPA-dependent alterations in leukocyte transcripts and proteins and illustrate the potential of peripheral leukocytes to serve as biological surrogates for CNS disease and therapy.

2. Materials and methods

2.1. Chemicals

All chemicals were obtained from Sigma–Aldrich Corp. (St. Louis, MO) and were of molecular biology grade or better unless otherwise noted. Cell culture reagents (e.g. RPMI-1640) were obtained from GIBCO/Invitrogen (Carls-