Neurobiology of Aging 43 (2016) 1–12

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Medium-chain plasma acylcarnitines, ketone levels, cognition, and gray matter volumes in healthy elderly, mildly cognitively impaired, or Alzheimer's disease subjects

Domenico Ciavardelli [a,b], Fabrizio Piras [c,d], Ada Consalvo [e], Claudia Rossi [e], Mirco Zucchelli [e], Carmine Di Ilio [e], Valerio Frazzini [b], Carlo Caltagirone [c,f], Gianfranco Spalletta [c,1], Stefano L. Sensi [b,g,h,1,*]

[a] School of Human and Social Science, "Kore" University of Enna, Enna, Italy
[b] Molecular Neurology Unit, Center of Excellence on Aging and Translational Medicine (Ce.S.I.-MeT), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy
[c] Department of Clinical and Behavioral Neurology, Neuropsychiatry Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy
[d] "Enrico Fermi" Centre for Study and Research, Rome, Italy
[e] Department of Medical, Oral, and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy
[f] Department of Neuroscience, University "Tor Vergata", Rome, Italy
[g] Department of Neurology, and Institute for Memory Impairments and Neurological Disorders, University of California-Irvine, Irvine, CA, USA
[h] Department of Pharmacology, and Institute for Memory Impairments and Neurological Disorders, University of California-Irvine, Irvine, CA, USA

ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form 2 February 2016
Accepted 6 March 2016
Available online 15 March 2016

Keywords:
Medium-chain acylcarnitines
Ketogenesis
Targeted metabolomics
Fatty acid beta-oxidation
Cognitive decline

ABSTRACT

Aging, amyloid deposition, and tau-related pathology are key contributors to the onset and progression of Alzheimer's disease (AD). However, AD is also associated with brain hypometabolism and deficits of mitochondrial bioenergetics. Plasma acylcarnitines (ACCs) are indirect indices of altered fatty acid beta-oxidation, and ketogenesis has been found to be decreased on aging. Furthermore, in elderly subjects, alterations in plasma levels of specific ACCs have been suggested to predict conversion to mild cognitive impairment (MCI) or AD. In this study, we assayed plasma profiles of ACCs in a cohort of healthy elderly control, MCI subjects, and AD patients. Compared with healthy controls or MCI subjects, AD patients showed significant lower plasma levels of several medium-chain ACCs. Furthermore, in AD patients, these lower concentrations were associated with lower prefrontal gray matter volumes and the presence of cognitive impairment. Interestingly, lower levels of medium-chain ACCs were also found to be associated with lower plasma levels of 2-hydroxybutyric acid. Overall, these findings suggest that altered metabolism of medium-chain ACCs and impaired ketogenesis can be metabolic features of AD.

1. Introduction

Aging, amyloid deposition, and tau-related pathology are key contributors to the onset and progression of Alzheimer's disease (AD; Herrup, 2010). Impaired energy metabolism has also been associated with brain aging and suggested to favor AD (Costantini et al., 2008; Mosconi et al., 2008). In that respect, compelling evidence indicates that age-dependent metabolic changes promote mitochondrial, endosomal-lysosomal, and peroxisomal dysfunctions. All these phenomena play a role in the development of AD–related neuronal loss and cognitive decline (Cataldo et al., 2000; Kou et al., 2011; Lin and Beal, 2006; Perez et al., 2015).

Glucose hypometabolism is an important early feature of the AD brain. Furthermore, several studies have indicated a direct correlation by which the occurrence of more severe degrees of hypometabolism can predict a more precipitous course of the disease (Cunnane et al., 2011; de Leon et al., 1983; Dukart et al., 2013; Frackowiak et al., 1981; Jack et al., 2010; Lehmann et al., 2013). Mechanisms involved in AD–related glucose hypometabolism are not completely known but evidence indicates that defective brain glucose transport, altered glycolysis, or amyloid beta–dependent deregulation of mitochondrial function as well as amyloid

* Corresponding author at: Molecular Neurology Unit, (CeSI-MeT), University "G. d'Annunzio", Via dei Vestini, 31, Chieti 66100, Italy. Tel.: +39 0871 541544; fax: +39 0871 541542.
E-mail address: ssensi@uci.edu (S.L. Sensi).
[1] Both authors have been acting as senior investigators and should be considered as equal last authors.

0197-4580/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2016.03.005

beta–mediated neurotoxicity participate in AD progression and development (Sullivan and Brown, 2005; Winkler et al., 2015). Chronic glucose hypometabolism also acts, indirectly, on the modulation of enzymatic activities that promote oxidative stress, another major contributing factor to AD pathogenesis (Mosconi et al., 2008).

Compelling evidence indicates that mitochondria play a pivotal role in AD–related hypometabolism (Chen and Zhong, 2013; Readnower et al., 2011). In preclinical AD models, mitochondrial deficits have been shown to precede the appearance of plaque deposition and neurofibrillary tangles, thereby supporting the idea that the organelle dysfunction is an early modulator of the AD–related pathologic cascade (Yao et al., 2009). In that respect, decreased expression or activity of several mitochondrial enzymes such as pyruvate dehydrogenase, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, and cytochrome C oxidase (Bubber et al., 2005) have been found in the AD brain. Similar findings have been shown in preclinical AD models (Ciavardelli et al., 2010; Rhein et al., 2009). Interestingly, AD–related mitochondrial deficits also occur outside of the central nervous system. In that regard, cytochrome C oxidase deficiency, decreased activity of alpha-ketoglutarate dehydrogenase, and altered mitochondrial fission and fusion have been described to occur in lymphocytes, platelets, and fibroblasts of AD patients (Leuner et al., 2012; Sorbi et al., 1983; Wang et al., 2008) as well as in skeletal muscles of preclinical AD models (Schuh et al., 2014).

Furthermore, it is conceivable that when standard energetic sources are falling behind, the AD brain is forced to rely on alternative energy substrates. In this view, ketone bodies may play an important role (Iadecola, 2015; VanItallie, 2015). Ketone bodies are produced by the catabolism of fatty acids, a process operated by liver mitochondria. Interestingly, functional deficits in hepatic mitochondrial and/or peroxisomal fatty acids metabolism have been indicated as potential contributors to AD–related cognitive impairment (Astarita and Piomelli, 2011). Moreover, in aging rats, decreased hepatic peroxisomal fatty acid beta-oxidation (FAO) has been linked to the development of alterations in the lipid composition of the brain (Yang et al., 2014).

Alterations of hepatic FAO can affect brain functioning. In that regard, ketone bodies are substrates that, in elderly subjects, may become an energetic source to be used, for brain functioning, as alternative to glucose. This concept is supported by findings indicating that healthy elderly brains increase consumption of energy substrates that are alternative to glucose when glucose utilization decreases, thereby suggesting a compensatory role played by these metabolites in coping with brain energy demand (Hoyer et al., 1991).

Plasma acylcarnitines (ACCs) are indirect indicators of hepatic FAO (Schooneman et al., 2013, 2015; Villarreal-Pérez et al., 2014). Furthermore, free carnitine and ACCs play a significant role in the modulation of brain metabolism (Jones et al., 2010). Of note, specific plasma ACCs have been found to be decreased in healthy elderly subjects converting to mild cognitive impairment (MCI) or AD (Mapstone et al., 2014).

In this study, we evaluated plasma ACC levels in a cohort of AD patients, MCI subjects, and age-matched healthy controls (HC). Correlations between levels of ACCs, plasma 2-hydroxybutyric acid (b-HBA), and degrees of cognitive status, assessed with the mini-mental state examination (MMSE), were also evaluated in the 3 study groups. Finally, structural changes occurring in brains of the 3 cohorts were investigated with voxel-based morphometry (VBM). These structural volumes were then correlated with ACC levels.

2. Materials and methods

2.1. Participants and ethics committee

A cohort of 107 individuals of whom 35 were experiencing probable AD, 38 had a diagnosis of MCI, and 34 were healthy-matched volunteers was enrolled for this study. AD and MCI patients were recruited by the memory clinic of the Santa Lucia Foundation (Rome, Italy). All individuals recruited as eligible for the study went through a clinical examination, neuropsychological testing, and Magnetic Resonance Imaging (MRI). Subjects gave written informed consent to a research protocol approved by the Joint Ethics Committee of the Santa Lucia Foundation.

HC were recruited through local advertisements and were screened using an extensive neuropsychological test battery to exclude subjects with dementia or MCI. To obtain a global index of cognitive impairment, we used the MMSE (Folstein et al., 1975). MMSE scores were adjusted for age and education level (Measso et al., 1993). The instrument is brief and easy to administer and is widely used to screen for cognitive deterioration (Tsoi et al., 2015). Subjects were also asked to perform the multiple features targets cancellation task (Gainotti et al., 2001), a test that assesses visuo-spatial explorative abilities and psychomotor processing speed, the immediate visual memory task (Gainotti et al., 1978), and the Benton face recognition test (Benton et al., 1983). Moreover, we administered the copy and delayed recall of Rey-Osterrieth's complex picture test (CROP and ROPR, respectively; Osterrieth, 1944) and the freehand copying of drawings with and without landmarks (Gainotti et al., 1977) to evaluate visual perception/constructional praxis, perceptual organizational skills, planning, and problem-solving. We also chose several tests from the mental deterioration battery (MDB; Carlesimo et al., 1996) to provide information about functioning of different cognitive domains such as language (MDB Sentence Construction), verbal memory (MDB Rey's 15-word immediate recall and delayed recall), logical reasoning (MDB Raven's Progressive Matrices' 47), and language (MDB phonological and semantic verbal fluency). Finally, set-shifting, cognitive flexibility, and resistance to interference were assessed using the modified Wisconsin card sorting test (Heaton et al., 1999). None of the HC subjects showed signs of cognitive deficits (Table 1, Supplementary Table 1).

In the MCI group, diagnosis of amnestic multi-domain MCI was made by trained neurologists who interviewed patients and next-of-kin (Petersen and Morris, 2005). Adopted criteria for MCI were as follows: (1) subjective memory impairment confirmed by a score below the cut off in at least 1 episodic memory test of the MDB or presence of impairment in cognitive areas other than memory (Supplementary Table 1); (2) lack of fulfillment of the National Institute of Health/National Institute on Aging criteria for dementia (McKhann et al., 2011); (3) presence of normal scores on instrumental activities of daily living and a total clinician dementia rating scale score of 0.5, consistently with minimal impairment in daily living activities; (4) lack of any evidence and clinical signs of neurologic and psychiatric disorders that could be responsible for memory deficits; (5) MRI scans lacking signs of focal lesions as computed according to the semi-automated method recently published by our group (Iorio et al., 2013; minimal diffuse changes or minimal lacunar lesions of white matter (WM) were, however, allowed); and (6) absence of signs of moderate-to-severe depression and/or anxiety as confirmed by scores on Beck's depression inventory and Hamilton anxiety rating scale (14 was the cut off score for both scales).

AD patients met clinical criteria established by the National Institute of Health/National Institute on Aging and the Alzheimer's Association (McKhann et al., 2011). According to these criteria, we