Experimental Gerontology 65 (2015) 69–78

Contents lists available at ScienceDirect

Experimental Gerontology

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

Hypercholesterolemia and neurodegeneration. Comparison of hippocampal phenotypes in LDLr knockout and APPswe/PS1dE9 mice

Miren Ettcheto [a,c], Dmitry Petrov [a,c], Ignacio Pedrós [b,c], Luisa de Lemos [a,c], Mercè Pallàs [a,c], Marta Alegret [a,d], Juan Carlos Laguna [a,d], Jaume Folch [b,c,1], Antoni Camins [a,c,e,*,1]

[a] Unitat de Farmacologia i Farmacognòsia Facultat de Farmàcia, Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain
[b] Unitat de Bioquímica, Facultat de Medicina i Ciències de la Salut, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat Rovira i Virgili, C./St. Llorenç 21, 43201 Reus, Tarragona, Spain
[c] Biomedical Research Networking Center in Neurodegenerative Diseases (CIBERNED), Madrid, Spain
[d] Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición, Spain
[e] Centro de Biotecnología, Universidad Nacional de Loja, Av. Pío Jaramillo Alvarado y Reinaldo Espinosa, La Argelia, Loja, Ecuador

ARTICLE INFO

Article history:
Received 1 January 2015
Received in revised form 16 March 2015
Accepted 18 March 2015
Available online 20 March 2015

Section Editor: Christian Humpel

Keywords:
Alzheimer's disease
Cholesterol
Hippocampus
APP/PS1
LDLR−/−
OXPHOS

ABSTRACT

Previous studies suggest that Alzheimer's disease (AD) neurobiology could not be explained solely by an increase in β-amyloid levels. Recently, it has been proposed that alterations in brain cholesterol metabolism may contribute to the pathogenesis of AD. In the present work, we focus on early changes in the hippocampal phenotypes of two mouse models in which cognitive impairments were previously described: a) the hypercholesterolemic LDL receptor knockout (LDLr −/−) and b) the APPswe/PS1dE9 (APP/PS1) transgenic model of familial AD. Our initial analysis, subsequent validation and additional experiments at the mRNA and protein levels demonstrate some parallels between the hippocampal phenotypes of these 2 mouse models, however our data suggest that the molecular mechanisms leading to cognitive decline are distinct in LDLr −/− and APP/PS1 animals. Genes related to cytokine signaling were significantly down-regulated in LDLr −/− mice when compared to both the wild-type and APP/PS1 mice, and these include prostaglandin-endoperoxide synthases 1 and 2 (*ptgs1* and *2*) and nerve grow factor (*ngf*). We have also detected reduced expression of genes related to lipid metabolism in LDLr −/− mice: peroxisome proliferator activated receptor gamma (*pparg*), pro-opiomelanocortin-alpha (*pomc*) and of protein kinase, AMP-activated, alpha 1 catalytic subunit of AMPK (*prkaa1*). Our array data also indicate that transcriptional activity of early genes involved in memory process, such as FBJ osteosarcoma oncogene (Fos) and the activity regulated cytoskeletal-associated protein (Arc) gene, are increased in the hippocampus of LDLr −/− mice. Several proteins like insulin degrading enzyme (IDE), PGC-1α, OXPHOS 1, NMDAR1 and cyclic AMP response element binding protein (CREB) are up-regulated in the LDLr −/− mice, while in the APP/PS1 mouse model only OXPHOS complexes 2, 3 and 5 are slightly downregulated. Further studies are necessary to understand the molecular pathways involved in memory loss in hypercholesterolemic LDLr −/− mice.

1. Introduction

Alzheimer's disease (AD) is one of the most common causes of senile dementia in the world. The disease was first described in the early twentieth century by Alois Alzheimer (Goedert and Ghetti, 2007). According to the classical hypothesis, a significant increase in brain β-amyloid levels causes the formation of senile plaques which is one of the two main markers of AD, together with hyperphosphorylated tau-protein-dependent neurofibrillary tangles (Hardy and Higgins, 1992). Until recently, it was widely accepted that an increase in cerebral soluble β-amyloid levels in general and soluble β_{1-42} in particular, is the principal contributor to AD progression (Hardy and Allsop, 1991; Swerdlow et al., 2010; Beauchet et al., 2014; Ferreira and Klein, 2011). However, success with potential therapeutic substances that inhibit the generation of beta amyloid has been low (Beauchet et al., 2014). This has resulted in a renewed interest in the search for alternative hypotheses to explain the underlying causes of the disease. For example, it has been suggested that AD may share many of the characteristics of peripheral metabolic disorders, including alterations in brain insulin

Abbreviations: FAD, familial Alzheimer's disease; LDLr, low-density lipoprotein receptor; ApoE, Apolipoprotein E; APP, amyloid precursor protein; ROS, reactive oxygen species; OXPHOS, oxidative phosphorylation; NRF, nuclear respiratory factors; PGC-1α, peroxisome proliferator activator receptor gamma-coactivator 1α; TFAM, mitochondrial transcription factor A; NMDA, N-methyl-D-aspartate; ARC, activity-regulated cytoskeleton-associated protein; CREB, cAMP response element-binding protein; IDE, insulin degrading enzyme; nNOS, neuronal nitric oxide synthase.

* Corresponding author at: Unitat de Farmacologia i Farmacognosia, Facultat de Farmàcia, Universitat de Barcelona, Spain, Avda/Diagonal 643, E-08028 Barcelona, Spain.
E-mail address: camins@ub.edu (A. Camins).
[1] Senior co-authors.

http://dx.doi.org/10.1016/j.exger.2015.03.010
0531-5565/

signaling and cholesterol metabolism abnormalities (Ferreira and Klein, 2011; Merlo et al., 2010; de la Monte and Tong, 2014).

AD etiology is complex, and it seems reasonable to propose that an increase in brain β-amyloid levels is unlikely to account for all aspects of the disease (Pimplikar, 2009; Pimplikar et al., 2010; Demetrius and Driver, 2013). Regarding the relationship between AD and metabolism, the fact that metabolic syndrome constitutes an independent risk factor for AD reinforces the hypothesis that the disease neuropathology is likely related to both insulin signaling and energy metabolism impairments (Pimplikar, 2009; Pimplikar et al., 2010; Moreira et al., 2013; Hiltunen et al., 2012; Ramos-Rodriguez et al., 2014; de la Monte, 2014).

Cholesterol metabolism deficiencies have been linked to atherosclerosis, vascular events, and AD. It is of interest that hypercholesterolemia may be a risk factor for mild cognitive impairments and dementia (Katsouri et al. 2011; Gamba et al., 2012; Moreira et al., 2014). Furthermore, altered cholesterol metabolism and hypercholesterolemia appear to play fundamental roles in amyloid plaque formation and tau hyperphosphorylation (Gamba et al., 2012).

The brain is rich in cholesterol and substantial in vitro evidence and experimental animal studies indicate that cholesterol levels in the brain affect the synthesis, clearance, and toxicity of Aβ (Reed et al., 2014; Moreira et al., 2012; Basak et al., 2012). The low-density lipoprotein receptor (LDLr) is involved in the regulation of Apolipoprotein E (ApoE) levels both in the periphery and in the central nervous system. ApoE, a cholesterol carrier associated with atherosclerosis, is a major risk factor for AD (Basak et al., 2012). However, the exact mechanisms underlying this phenomenon remain unknown, as plasma cholesterol is not able to cross the blood–brain barrier (Moreira et al., 2012). LDLr has been identified on astrocytes and a number of studies show that it modulates amyloid deposition in transgenic mouse models of AD (Katsouri and Georgopoulos, 2011; Basak et al., 2012). Previous studies in LDLr-deficient mice have shown that LDLr regulates glial response independently of ApoE and that it plays a role in amyloid deposition (Katsouri and Georgopoulos, 2011; Basak et al., 2012). An LDLr knockout (LDLr −/−) model has been originally developed to study atherosclerosis, and the physiological parameters of these mice are comparable to those found in familial hypercholesterolemia in humans, making this model an attractive candidate to study the impact of high circulating cholesterol levels in various tissues (Ishibashi et al., 1993). In the CNS, the lack of LDLr causes an increase in brain ApoE levels, but it has no effect on hippocampal or CSF cholesterol (Fryer et al., 2005). In addition, it has been shown that LDLr −/− mice display a reduced number of proliferating cells and presynaptic boutons in the hippocampus, potentially contributing to cognitive impairment (Mulder et al., 2004, 2007). Thus, experiments carried out with these mice link hypercholesterolemia to cognitive dysfunction, potentially mediated by increased neuroinflammation and aberrant amyloid precursor protein (APP) processing (Thirumangalakudi et al., 2008). Furthermore, hypercholesterolemia accelerates β-amyloid accumulation and tau pathology upon intracerebral injection of $A\beta_{25-35}$ in AD-like mice, which is accompanied by microglial activation and subsequent aggravation of memory impairment (Park et al., 2013). Hypercholesterolemia also triggers an oxidant–antioxidant imbalance resulting in excessive production of reactive oxygen species (ROS), particularly in the prefrontal cortex, causing oxidative damage together with alterations in cholinergic signaling, affecting the processes of learning and memory (Thirumangalakudi et al., 2008; de Oliveira et al., 2011, 2013, 2014). The mitochondrion is the main source of ROS, and several studies clearly show increased ROS production when the respiratory chain is inhibited, as observed in mitochondrial diseases or in experimental models of oxidative phosphorylation (OXPHOS) deficiencies (de Oliveira et al., 2013, 2014).

The precise nature of mechanisms leading to cognitive loss induced by hypercholesterolemia is an area of active research. Evola et al. (2010), using ApoE knockout mice (another widely used model of hypercholesterolemia), proposed that an increase in inflammatory cytokines in the cerebral vascular network could be involved (Evola et al., 2010). An oxidative–inflammatory cycle within the vascular bed could have deleterious consequences for brain function and cognition. In line with this hypothesis, Hafezi-Moghadam et al. (2007) showed that the genetic deletion of ApoE induces a destabilizing effect on the cerebral microcirculation in aged hypercholesterolemic mice, causing blood–brain barrier leakage (Hafezi-Moghadam et al., 2007). Failure of the blood–brain barrier, with leakage of serum components into and through the walls of small cerebral vessels, can lead to neuronal and glial damage with persistent activation of microglia and astrocytes, which may be responsible for cognitive impairment (Rapp et al., 2008).

Hyperlipidemia, hypercholesterolemia, and obesity have all been described as contributing factors to the disease progression in mouse models of AD (Ramos-Rodriguez et al., 2014). The APPswe/PS1dE9 double transgenic mouse (APP/PS1) model was designed to mimic human familial AD pathology. In APP/PS1 animals, two strategies are combined to reach elevated cerebral Aβ levels: targeted overexpression of the mutant human form of APP, together with the mutant presenilin-1 (PS1), which results in aberrant amyloid protein processing (Ramos-Rodriguez et al., 2014; Pedrós et al., 2014; Aso et al., 2012). In the current work, we compare hippocampal phenotypes of 6-month-old APP/PS1 and LDLr −/− mice in order to identify potential mechanisms leading to cognitive decline at the molecular level.

2. Materials and methods

2.1. Animals

In this study, LDL receptor-deficient knockout (LDLr −/−), APPswe/PS1dE9 (APP/PS1) double transgenic, and wild-type control mice were used. All three models have C57BL/6J genetic background. In order to avoid possible variables related to hormonal changes that may occur with females, only the male mice we used. LDLr −/− and APP/PS1 animals were obtained from Jackson Laboratory (B6.129S7-$Ldlr^{tm1Her}$/J), stock number: 002207; and (B6.Cg-Tg(APP695)3Dbo Tg(PSEN1dE9)S9Dbo/Mmjax), stock number: 005866.

APP/PS1 animals co-express a Swedish (K594M/N595L) mutation of a chimeric mouse/human APP-695 (Mo/HuAPP695swe), together with the human exon-9-deleted variant of PS1 (PS1-dE9) under control of a mouse prion protein promoter, allowing these mice to secrete elevated amounts of human Aβ peptide. The APPswe mutation is a favorable substrate for β-secretase, whereas the PS1dE9 mutation alters γ-secretase cleavage, thereby promoting overproduction of Aβ42. Animals were kept under controlled temperature, humidity and light conditions with food and water provided ad libitum. Mice were treated according to the European Community Council Directive 86/609EEC and the procedure established by the *Departament d'Agricultura, Ramaderia i Pesca* of the *Generalitat de Catalunya*. Every effort was made to minimize animal suffering and to reduce the number of animals used.

Forty animals divided into three groups were used for the present study: 11 wild-type, 14 APP/PS1 and 15 LDLr −/− mice of six months of age.

2.2. Peripheral metabolic tests

Plasma cholesterol was determined with the Cholesterol CHOD-POD colorimetric test (ref. 1001091; Spinreact, Girona, Spain); blood glucose levels with an Accutrend Glucose meter (ref. 11447475; Roche Diagnostics); plasma leptin with the RIA kit (ref. RL-83K; Millipore, Bedford, MA, USA); and plasma insulin with the mouse Insulin ELISA Kit (ref. EZRMI-13K; Millipore, Bedford, MA, USA); in accordance with the manufacturers' protocols (Table 1).

2.3. Western blot analysis

Aliquots of hippocampus homogenate containing 15 μg of protein per sample were analyzed using the Western blot method. In brief,