Neurobiology of Aging xxx (2014) 1–11

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Pinocembrin improves cognition and protects the neurovascular unit in Alzheimer related deficits

Rui Liu [a], Jin-ze Li [a], Jun-ke Song [a], Dan Zhou [a], Chao Huang [a], Xiao-yu Bai [a], Tao Xie [a], Xue Zhang [a], Yong-jie Li [a], Cai-xia Wu [a,b], Lan Zhang [c], Lin Li [c], Tian-tai Zhang [a,**], Guan-hua Du [a,*]

[a] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, P. R. China
[b] School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, China
[c] Key Laboratory for Neurodegenerative Diseases of Ministry of Education, Department of Pharmacology, Xuanwu Hospital of Capital Medical University, Beijing Geriatric Medical Research Center, Beijing 100053, PR China

ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form 13 November 2013
Accepted 25 December 2013

Keywords:
Alzheimer's disease
Amyloid-β peptide
The neurovascular unit
Pinocembrin
The receptor for advanced glycation end products

ABSTRACT

Amyloid-β (Aβ) peptides accumulate in the brain and initiate a cascade of pathologic events in Alzheimer's disease. The receptor for advanced glycation end products (RAGE) has been implicated to mediate Aβ-induced perturbations in the neurovascular unit (NVU). We demonstrated that pinocembrin exhibits neuroprotection through inhibition of the Aβ and/or RAGE pathway, but the therapeutic role and mechanism involved are not ascertained. Here, we report that a 3-month treatment with pinocembrin prevents the cognition decline in APP/PS1 transgenic mice without altering Aβ burden and oxidative stress. Instead, pinocembrin is effective in conferring neurovascular protection through maintenance of neuropil ultrastructure, reduction of glial activation and levels of inflammatory mediators, preservation of microvascular function, improving the cholinergic system by conserving the ERK-CREB-BDNF pathway, and modulation of RAGE-mediated transduction. Furthermore, in an in vitro model, pinocembrin provides the NVU protection against fibrillar $A\beta_{1-42}$, accompanied by regulation of neurovascular RAGE pathways. Our findings indicate that pinocembrin improves cognition, at least in part, attributable to the NVU protection, and highlights pinocembrin as a potential therapeutic strategy for the prevention and/or treatment of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, characterized by abnormal accumulation of amyloid plaques and neurofibrillary tangles throughout the cerebrocortical and limbic regions (Coyle et al., 1983). Recent studies have provided a vascular-derived insult hypothesis that AD is not solely caused by abnormal amyloid metabolism, but blood vessel damage must also be involved (Marchesi et al., 2011; Shin et al., 2007). Further evidence suggests that initial vascular damage plays a pivotal role in functional and structural changes of neurons (Bell et al., 2010; Marchesi et al., 2011; Zlokovic, 2005), and accumulation of brain amyloid-β (Aβ) peptides is subsequent to the blood-brain barrier (BBB) dysfunction and reduction in cerebral blood flow (Bell et al., 2010; Zlokovic, 2011). Importantly, vascular clearance of brain Aβ is impaired after BBB damage (Deane et al., 2004, 2003; Zlokovic, 2011) and the β-amyloidogenic process is up-regulated as a result of reduced brain blood perfusion (Cullen et al., 2006; Kumar-Singh et al., 2005; Weller et al., 2008). At subsequent pathophysiological events, Aβ accelerates neurovascular (Deane et al., 2003; Zlokovic, 2005) and neuronal dysfunction (Takuma et al., 2009; Walsh et al., 2002; Yan et al., 1996), causes oxidative stress, and mediates chronic inflammatory processes, which are involved in the neurovascular unit (NVU) pathology.

The NVU comprises of cerebral blood vessels and surrounding astrocytes, neurons, and other supporting cells (e.g., perivascular microglia and pericytes) (Iadecola, 2004), which couples local neuronal function to local cerebral blood flow, as well as regulating

* Corresponding author at: State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, P. R. China. Tel./fax: +86 10 63165184.
** Alternate corresponding author at: State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, P. R. China. Tel./Fax: +86 10 63165184.
E-mail addresses: ttzhang@imm.ac.cn (T.-t. Zhang), bessie0420@163.com (G.-h. Du).

0197-4580/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.12.031

transport of blood-borne molecules across the BBB (Bell and Zlokovic, 2009; Kalaria, 2009; Zlokovic, 2008). The intimate association between astrocytes and brain microvessels is referred to as the major component of the NVU; each cell type is important for the physiological function of the BBB (Hawkins and Davis, 2005), which restricts permeability across brain endothelium (Zloković et al., 1985a, 1985b) and regulates the transport of nutrients and peptides using specific transporters expressed in brain endothelium either under physiological or pathologic conditions (Zlokovic and Apuzzo, 1997; Zlokovic et al., 1990, 1989). The BBB is easily disrupted under pathologic conditions (Sagare et al., 2013; Sengillo et al., 2013). Animals receiving a peripheral Aβ injection (Liu et al., 2011a; Su et al., 1999) and transgenic AD mice (Ujiie et al., 2003) displayed increased BBB permeability. Further, morphologic alterations of tight junction proteins, suggestive of a leaky BBB, have been observed in AD patients' brain biopsies (Stewart et al., 1992). As the therapy of reducing neuronal injury alone would be expected to be less successful in slowing the course of AD, the treatment strategy with maintaining the integrity of BBB is recommended as a potential method to prevent degeneration of this disease.

In the NVU, the receptor for advanced glycation end products (RAGE), addressed as a neurovascular coupling molecule (Zlokovic et al., 2008), plays an important role as a cell-surface receptor for different forms of Aβ at the BBB, neurons, and microglia (Deane et al., 2012; Zlokovic, 2011). RAGE mediates the re-entry of circulating Aβ into the brain at the BBB, followed by pathologic events in the endothelium, such as NF-κB-mediated activation accompanied with inflammatory cytokine production (Liu et al., 2013). RAGE ligation in neurons and glia has been shown to activate multiple signaling pathways, including the Ras-extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 MAP kinase (p38 MAPK) pathways. This results in oxidative stress, inflammation, cholinergic dysfunction, and neurovascular uncoupling. Because of its pivotal pathologic role in neurovascular signaling and potential cytoprotection toward the NVU through interference, RAGE pathway is increasingly considered as a new therapeutic approach for AD.

Pinocembrin (5, 7-dihydroxyflavanone, Fig. 1A) is a flavonoid abundant in propolis and extracted as a pure compound. Pinocembrin has shown to be effective in the protection of brain injury from ischemic and Aβ impairment. It was approved by the State Food and Drug Administration of China for treatment of stroke in 2008. We showed that pinocembrin protected against ischemic injury and reduced the area of cerebral infarction in ischemia models (Gao et al., 2010; Liu et al., 2008; Meng et al., 2011; Shi et al., 2011). Moreover, pinocembrin has been investigated for the ability to express NVU protection by decreasing oxidative damage (Shi et al., 2011) and inhibiting inflammatory responses (Gao et al., 2010; Liu et al., 2012). Recently, we found that pinocembrin alleviated cognitive deficits in the vascular dementia model (Guang and Du, 2006) and intracerebroventricular Aβ-injected model (Liu et al., 2012). In the $A\beta_{1-42}$-treated RAGE overexpressing cell model, pinocembrin inhibited the overexpression of RAGE (Liu et al., 2012). Moreover, pinocembrin attenuated neuronal apoptosis through down-regulating RAGE expression and inhibiting RAGE downstream pathways, both in SH-SY5Y cells overexpressing the Swedish mutant form of human Aβ-precursor protein (APP) and in the $A\beta_{25-35}$-induced amnesia model (Liu et al., 2012). These results suggest that pinocembrin has potential therapeutic effects on Aβ-related cognitive deficits and might have prospects as an AD therapeutic agent.

To confirm the cognitive improvement and explore the potential mechanism, we examined the effects of pinocembrin in the APP and presenilin 1 (PS1) double transgenic AD mouse model on cognitive impairment and pathologic changes within the NVU. In addition, we investigated the mechanisms underlying the efficacy on potential signal transduction in an in vitro model.

2. Methods

2.1. Materials

Pinocembrin (purity >99%) was synthesized by Institute of Materia Medica, Chinese Academy of Medical Sciences. Unless otherwise stated, all other reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Animals and drug treatment

APP/PS1 double transgenic mice were purchased from The Jackson Laboratory (strain name, B6C3-Tg [AβPPswe, PSEN1dE9] 85Dbo/J; stock number, 004462). Age-matched wild-type (WT) littermates were used as controls. All animals received care according to *the Guide for the Care and Use of Laboratory Animals*.

Four-month-old APP/PS1 mice and littermate WT mice were divided randomly into 4 groups: WT controls (n = 9, 4 males and 5 females), WT + pinocembrin 40 mg/kg (n = 9, 5 males and 4 females), APP/PS1 controls (n = 9, 5 males and 4 females), and APP/PS1 + pinocembrin 40 mg/kg (n = 9, 5 males and 4 females). Pinocembrin-treated WT and APP/PS1 groups received pinocembrin dissolved in 20% hydroxypropyl-β-cyclodextrin by oral gavage 5 days per week. The vehicle controls received 20% hydroxypropyl-β-cyclodextrin in the same manner. Treatment continued for 12 weeks.

After the behavioral tests were completed, the mice were divided into 3 parallel experiments: (1) one-third of each group was anaesthetized and blood was collected followed by transcardial perfusion with phosphate buffered saline. The brains were quickly removed. One hemi-brain was snap frozen in liquid nitrogen. The other was fixed and stored in 4% paraformaldehyde in phosphate buffered saline and later dehydrated. The effects of pinocembrin on cholinergic neuronal changes and protein expressions were examined; (2) another one-third mice were used for BBB permeability assessment and neuropil ultrastructure detection; and (3) the remainders were used for the detection of cerebral oxidative stress, inflammatory mediator levels, and Aβ burdens. The division of mice into treatment groups and selection of mice to be sacrificed within each group were both done randomly.

2.3. Behavioral tests

Spatial learning and memory capabilities were assessed by Morris water maze (MWM) test. The acquisition task consisted of 5 consecutive days of training. The duration required to escape onto the hidden platform was recorded as escape latency. In the probe trial, the time the mice spent in the platform quadrant and numbers of crossings where the platform had been located were recorded. Only mice with swimming speed over 8 cm/s in all trials were included in the analysis to discard occasional floating mice. The passive avoidance task was used to assess the contextual short-term memory of the mice. The time a mouse took to enter the NON-illuminated compartment after opening the door was defined as latency.

2.4. Histochemistry

Slices from frontal cortex were used for staining with anti-glial fibrillary acidic protein (GFAP) (1:1000, Abcam, Cambridge, UK, USA), anti-ionized calcium-binding adaptor molecule 1 (Iba-1) (1:500, Wako, Osaka, Japan), and anti-p38 (1:200, Cell Signaling Technology, Beverly, MA, USA). Image analysis was performed using Image-Pro Plus (Media Cybernetics, Bethesda, MD, USA).