Alzheimer's & Dementia 12 (2016) 678-686

Featured Article

Selecting for neurogenic potential as an alternative for Alzheimer's disease drug discovery

Marguerite Prior[a,1], Joshua Goldberg[a,1], Chandramouli Chiruta[a], Catherine Farrokhi[b], Mariya Kopynets[a], Amanda J. Roberts[c], David Schubert[a,*]

[a]*The Salk Institute for Biological Studies, Cellular Neurobiology, La Jolla, CA, USA*
[b]*Behavior Testing Core, The Salk Institute for Biological Studies, La Jolla, CA, USA*
[c]*Department of Molecular and Cellular Neurosciences, The Scripps Research Institute, La Jolla CA, USA*

Abstract

Introduction: Neurons die in Alzheimer's disease (AD) and are not effectively replaced. An alternative approach to maintain nerve cell number is to identify compounds that stimulate the proliferation of endogenous neural stem cells in old individuals to replace lost neurons. However, unless a neurogenic drug is also neuroprotective, the replacement of lost neurons will not be sufficient to stop disease progression.
Methods: The neuroprotective AD drug candidate J147 is shown to enhance memory, improve dendritic structure, and stimulate cell division in germinal regions of the brains of very old mice. Based on the potential neurogenic potential of J147, a neuronal stem cell screening assay was developed to optimize derivatives of J147 for human neurogenesis.
Results: The best derivative of J147, CAD-031, maintains the neuroprotective and memory enhancing properties of J147, yet is more active in the human neural stem cell assays.
Discussion: The combined properties of neuroprotection, neurogenesis, and memory enhancement in a single drug are more likely to be effective for the treatment of age-associated neurodegenerative disorders than any individual activity alone.
© 2016 The Alzheimer's Association. Published by Elsevier Inc. All rights reserved.

Keywords: Alzheimer's disease; Neurodegenerative disease; Aging; Neurogenesis; Stem cells; Neuroprotection; Memory enhancement; J147

1. Introduction

Potential Alzheimer's disease (AD) therapies currently in clinical trials are primarily focused on the amyloid pathway and do not stimulate the replacement of dead cells in the aged human brain. Even if nerve cells could be replaced, the toxic environment will likely kill new cells unless they are protected. Therefore, drugs that stimulate neurogenesis alone will be ineffective. However, a drug that is both neurogenic and neuroprotective may hold more promise. With the advent of the ability to use human embryonic stem cell–derived (hES) neural precursor cells (hNPCs) as a screen to identify neurogenic compounds, it should be possible to tailor AD drug candidates that are both neurogenic and neuroprotective. This approach has not, however, been previously used to identify an AD drug candidate.

Our laboratory has developed a cell-based phenotypic screening paradigm that mimics multiple age-associated central nervous system (CNS) pathologies [1]. We have successfully identified a number of neuroprotective molecules, including a synthetic derivative of curcumin called J147. J147 facilitates memory in normal rodents and prevents the loss of synaptic proteins and cognitive decline when administered to 3-month-old APPswe/PS1ΔE9 mice for

D.S., M.P., and C.C. are consultants for Abrexa Pharmaceuticals, a virtual company working on the development of J147 for AD.

[1]These authors contributed equally to this work.

*Corresponding author. Tel.: +1 858-453-4100..
E-mail address: schubert@salk.edu

http://dx.doi.org/10.1016/j.jalz.2016.03.016
1552-5260/© 2016 The Alzheimer's Association. Published by Elsevier Inc. All rights reserved.

7 months [2]. Furthermore, it has the ability to reverse severe cognitive deficits when fed to very old AD mice [3]. Here, we demonstrate that J147 improved spatial memory using an assay that is widely used in the clinic, and we show that J147 reduced the loss of dendritic spines in 24-month-old C57Bl/6 mice. Importantly, J147 also stimulated the *in vivo* proliferation of cells in the dentate gyrus and NPCs within the subventricular zone (SVZ) neural stem cell niche, areas of the brain where new nerve cells are born.

As J147 stimulated cell division within the SVZ in old mice, we hypothesized that it might be possible to optimize J147 for human neurogenesis while maintaining its neuroprotective properties, thus increasing its therapeutic potential. We used a hES cell-derived hNPC screening paradigm in combination with phenotypic screening assays for old age–associated brain toxicities to synthesize CAD-031. CAD-031 maintains the neuroprotective properties of J147 but is more neurogenic in the hNPC screening assay. The combined properties of neuroprotection, neurogenesis, and memory enhancement in a single drug will likely be more effective than a single target compound for the treatment of age-related neurodegenerative disorders like AD that result from the convergence of multiple toxicities.

2. Methods

2.1. Animal studies

Animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. For the aging study, 24 male C57Bl/6 mice aged 24 months were obtained from the National Institute on Aging, Aged Rodent Resource and housed singly. The APPswe/PS1ΔE9 transgenic mice (line 85) carry two transgenes, the mouse/human chimeric APP/Swe, linked to Swedish FAD and human PS1ΔE9 [2,3]. The J147 diet was prepared by the addition of J147 at 200 ppm, and control diet was the same food without J147. The Aricept diet was prepared by the addition of CAD-031 at 200 ppm and Aricept at 14 ppm. All behavioral assays have been extensively detailed in our laboratories [2,3]. To assay cell proliferation, 5-bromo-2′-deoxyuridine (BrdU) was injected i.p at a concentration of 100 mg/kg for 7 days 24 hours after the last BrdU injection mice were euthanized. Cells in the SVZ were co-stained for BrdU and doublecortin (DCX).

2.2. Immunoblotting and imaging

Tissue preparation, immunohistochemistry, and immunoblotting were done as previously described [2,3].

2.3. Golgi staining

Golgi staining was performed according to the manufacturer's instructions (FD NeuroTechnologies). Each dendritic branch was imaged with a through-focus series and stitched together using Axiovision software. The microscopy and computer software were calibrated to measure the length of dendritic branches. Dendritic spines were randomly selected and counted blind.

2.4. Neurogenesis screening assay

NPCs were made from human embryonic stem cell lines Hues6 and H9 using embryoid body procedures [4]. Experiments were designed so that the drug effect was compared to fibroblast growth factor-2 (FGF). Experimental conditions included +FGF, −FGF and −FGF in the presence of drug. Drugs were added at the concentrations and times indicated in the text. Cells were harvested at the time points indicated for qPCR analysis using published primers. Each sample was analyzed in triplicate. Relative gene expression values were analyzed using the SDS software package (version 2.3). GAPDH served as the housekeeping gene, and genes were normalized to GAPDH before comparisons were made.

2.5. Neuroprotection assays

HT22 is a hippocampal nerve cell line that is killed by 5-mM glutamate via an oxidative stress-based cell death cascade. MC65 cells are killed by the conditional expression of Aβ. These assays were done as described [1–3].

2.6. Chemical synthesis and pharmacology

The synthesis of the J147 derivatives and the assays related to the pharmacological properties of CAD-031 are detailed in the Supplementary Material.

3. Results

3.1. J147 enhances memory and dendritic spine number in old mice

Initially, the behavioral effect of J147 was investigated in aged mice. Pattern separation is a memory assay used in humans to identify cognitive deficits critical to the encoding and retrieval of episodic memories that become reduced with age [5,6]. Twenty-four–month-old male C57Bl/6 mice were treated for 6 months with either J147 chow or normal chow without drug, using 8-month-old mice as controls. Although both young and old animals recognized when an object was moved a large distance (135°), a reduction in the recognition index (RI) in aged mice was observed when the object was moved a smaller distance of 45°. The reduction in the RI was reversed on treatment with J147 (Fig. 1A).

Anxiety is another old age–associated behavior that can be assayed using the Elevated Plus Maze (EPM). Young and old mice spent similar amounts of time in the open arms; however, old mice displayed a significant reduction in exploratory activity as evidenced by the reduction in arm entries (Fig. 1B-C). J147 significantly increased the time spent in the open arms in aged mice but did not rescue

Download English Version:

https://daneshyari.com/en/article/5623832

Download Persian Version:

https://daneshyari.com/article/5623832

Daneshyari.com