Neurobiology of Aging xxx (2013) 1–13

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

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

Phosphodiesterase 7 inhibitor reduced cognitive impairment and pathological hallmarks in a mouse model of Alzheimer's disease

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ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form 10 January 2013
Accepted 11 March 2013

Keywords:
Alzheimer's disease
Phosphodiesterase inhibitors
Aβ degradation
Cognition
Astrocytes
Transgenic mice

ABSTRACT

Elevated levels of amyloid beta (Aβ) peptide, hyperphosphorylation of tau protein, and inflammation are pathological hallmarks in Alzheimer's disease (AD). Phosphodiesterase 7 (PDE7) regulates the inflammatory response through the cyclic adenosine monophosphate signaling cascade, and thus plays a central role in AD. The aim of this study was to evaluate the efficacy of an inhibitor of PDE7, named S14, in a mouse model of AD. We report that APP/Ps1 mice treated daily for 4 weeks with S14 show: (1) significant attenuation in behavioral impairment; (2) decreased brain Aβ deposition; (3) enhanced astrocyte-mediated Aβ degradation; and (4) decreased tau phosphorylation. These effects are mediated via the cyclic adenosine monophosphate/cyclic adenosine monophosphate response element-binding protein signaling pathway, and inactivation of glycogen synthase kinase (GSK)3. Our data support the use of PDE7 inhibitors, and specifically S14, as effective therapeutic agents for the prevention and treatment of AD.

1. Introduction

Pathogenesis of Alzheimer's disease (AD) is characterized by accumulation of amyloid beta (Aβ) peptides in the brain, intracellular neurofibrillary tangles containing hyperphosphorylated tau, and synapse dysfunction that all lead to the death of brain neurons and to progressive memory loss in patients (Selkoe, 2001). Chronic neuroinflammation is also a pathological hallmark of AD, manifested by activated microglia and reactive astrocytes (Akiyama et al., 2000; Bornemann et al., 2001; Matsuoka et al., 2001). Accumulation of Aβ can trigger activation of glial cells, which will set off an inflammatory response that, over time, becomes chronic causing a persistent deleterious condition (Akiyama et al., 2000). The role of neuroinflammation in the development and progression of AD is, however, not clear. Microglia and astrocytes secrete inflammatory cytokines, chemokines and neurotoxins on activation, and can thereby promote neuronal degeneration. However, activated astrocytes surrounding Aβ plaques might have beneficial effects through phagocytosis, and thus elimination, of Aβ (Bard et al., 2000; Schenk et al., 1999). Astrocytes have also been reported to be able to migrate toward Aβ plaques and, on contact, to degrade Aβ (Koistinaho et al., 2004; Wyss-Coray et al., 2003).

Traditional anti-inflammatory therapies such as nonsteroidal anti-inflammatory drugs have produced conflicting results in AD, highlighting the need for new, more specific anti-inflammatory targets (Imbimbo et al., 2010). Diverse studies have suggested that cyclic adenosine monophosphate (cAMP) levels might play an important role in neuroprotection and in neuroinflammatory response (Lonze et al., 2002; Volakakis et al., 2010). cAMP is degraded by the action of cyclic nucleotide 3′,5′-phosphodiesterases (PDEs) (Conti and Beavo, 2007; Mehats et al., 2002). Selective inhibitors of cAMP-specific PDEs have recently emerged as new therapeutic agents for the treatment of human diseases including disorders of the central nervous system (CNS) (Menniti et al., 2006). More than 11 isoenzymes with different splicing variants of the PDE family have been identified to date (Conti and Beavo, 2007). Among them, PDE4, PDE7 and PDE8 are cAMP-specific PDEs. The PDE7

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http://dx.doi.org/10.1016/j.neurobiolaging.2013.03.011

family is composed of 2 genes, *PDE7A* and *PDE7B*, and is expressed in the brain and peripheral tissues. Specific inhibitors of PDE7 have been recently reported as potential new drugs for the treatment of brain diseases (Gil et al., 2008).

Recent studies from our research group have reported the pharmacological properties of a new chemical family of small molecules with a central heterocyclic core of quinazoline which act as PDE7 inhibitors (Castaño et al., 2009). The therapeutic potential of some of them has been recently reported in a stroke model (Redondo et al., 2012). Moreover, the quinazoline named S14, is a cell-permeable PDE7 inhibitor (Castro et al., 2008), able to cross the blood–brain barrier, which has a neuroprotective profile against different insults, including spinal cord injury (Paterniti et al., 2011), and cellular and rodent models of Parkinson disease (Morales-Garcia et al., 2011). Here, we examined the specific profile of S14 on different PDEs, its genotoxicity safety and in vivo pharmacokinetics, together with the effect of S14 treatment on the neurodegenerative pathology in APP/Ps1 mice, a well established model for AD. When administered systemically to 5-month-old APP/Ps1 mice for 4 weeks, S14 reduced behavioral impairment, brain Aβ accumulation, tau phosphorylation, glial activation, and cell death. More intriguingly, we found that S14 treatment stimulated astrocyte-mediated Aβ degradation.

2. Methods

2.1. PDE7 inhibitors and PDEs activity determination

BRL50481, a well-known small-molecule inhibitor of PDE7, was purchased from Sigma-Aldrich. S14 was synthesized in the laboratories of Instituto de Quimica Medica-CSIC according to described procedures (Castro et al., 2008).

The methodology used for measuring PDEs activity was based on a Scintillation Proximity Assay from Perkin Elmer (TRKQ7090). The activity of *PDE3A*, *PDE4B*, *PDE4D*, *PDE7A*, *PDE7B*, *PDE9A*, and *PDE10A* was measured by coincubating the enzyme with [^{3}H]cAMP and the hydrolysis of the nucleotide was quantified by radioactivity measurement after binding of [^{3}H]adenosine monophosphate to scintillation binding beads. PDEs (0.02 U of each) were incubated in a 96-well flexiplate with 5 nCi of [^{3}H]cAMP and S14 in 100 μL of assay buffer (contained in the kit) for 20 minutes at 30 °C. After the incubation time, 1 mg of scintillation beads was added to each well and the plate was shaken for 1 hour at room temperature. Finally, beads were settled for 30 minutes and radioactivity was detected in a Microbeta Trilux reader. Half maximal inhibitory concentration (IC_{50}) values were calculated by nonlinear regression fitting using GraphPad Prism. Data (radioactivity vs. log concentration) was fitted to a sigmoidal dose–response equation: $Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10\text{^}((\log IC_{50} - X) \times n))$, where Bottom and Top represent the minimum and maximum inhibition for PDE, respectively, IC_{50} the concentration of S14 that inhibited the PDE activity at 50%, and n the slope of the concentration–response curve.

2.2. Pharmacokinetics studies of S14

To evaluate levels in plasma and in brain after intraperitoneal (i.p.) and oral (p.o.) S14 administration, 3 male Swiss albino mice were used per point. The administered dose was 10 mg/kg (i.p. and p.o.) and the dosing volume was 10 mL/kg in both cases. The formulation used was dimethyl sulfoxide (DMSO) (i.p.) and 0.5% wt/vol sodium carboxyl methyl cellulose and 0.1% vol/vol Tween 80 in water (p.o.). The study design was as follows: blood samples (approximately 60 μL) were collected from the retro-orbital plexus of each mouse. The plasma and brain samples were obtained at predose, 0.08, 0.25, 0.5, 1, 2, 4, 8, and 24 hours (i.p.) and predose, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours (p.o.) postdosing. Plasma was harvested by centrifugation of blood and stored at −70 °C until analysis. Immediately after collection of blood, brain samples were collected from each mouse. Tissue samples (brain) were homogenized using ice-cold phosphate buffered saline (pH 7.4) and homogenates were stored at less than −70 °C until analysis. Total homogenate volume was 3 times the tissue weight. Plasma and brain samples were quantified by the developed a solid-phase extraction LC-MS/MS method lower limit of quantization (LLOQ): 1.02 ng/mL. The plasma and brain concentration time data for S14 were used for the pharmacokinetic analysis. Brain concentrations were converted to ng/g from ng/mL considering total homogenate volume and brain weight (i.e., the dilution factor was 3). Pharmacokinetic analysis was performed using the NCA module of WinNonlin Enterprise Version 5.2

2.3. Mutagenicity assay

The method of direct incubation in plate using culture of *Salmonella typhimurium* TA98 strain was performed on S14 compound. The influence of metabolic activation was tested by adding the S9 fraction of mouse liver. Positive controls of nucleoside diphosphate (NDP) and 2-amino fluorene (2AF) were run in parallel. The revertant number was manually counted and compared with the natural revertant. The compound is considered mutagenic when the number of revertant colonies is at least 2-fold of the spontaneous reverting frequencies for at least 2 consecutive dose levels. The maximum assayed doses were determined according to toxic effect on *S. Typhimurium* previously determined for each compound.

2.4. Cell cultures

Primary neuronal and astrocyte cultures from the cerebral cortex and hippocampus were performed as previously described (Alvira-Botero et al., 2010). Primary cortical and hippocampal neurons were obtained from Wistar rat embryos on prenatal day 17, and primary cortical astrocytes from Wistar rat on postnatal day 3. Cultures were kept at 37 °C in a humidified atmosphere containing 5% CO_2 for 7 days before experimentation. Then, cultures were incubated in fresh medium with or without S14 (30 μM), alone or in combination with $A\beta_{1-42}$ (10 μM) and BRL50481 (30 μM). S14 was previously dissolved in DMSO, and then diluted in a sterile culture medium, as reported previously (Morales-Garcia et al., 2011). $A\beta_{1-42}$ was dissolved in acetic acid 0.1 M, and then was diluted in sterile distilled water as previously reported (Dietrich et al., 2008).

2.5. Animal experiments

Male double transgenic APP/Ps1 mice (5-month-old), a cross between Tg2576 (overexpressing human APP695) and mutant Ps1 (M146L), were used from our inbred colony (Instituto de Investigacion Hospital 12 de Octubre). Age-matched mice not expressing the transgene were used as wild type controls. Mice were treated intraperitoneally with vehicle (5% DMSO) or S14 (5 mg/kg daily) for 4 weeks. At the end of treatment, animals were deeply anesthetized and transcardially perfused either with saline for biochemical analysis, or 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4 for immunohistochemical analysis. All animals were handled and cared for according to the Council Directive 2010/63/UE of 22 September 2010.

2.6. Behavioral testing

After adaptation to human handling, behavioral tests were conducted in APP/Ps1 and wild type nontransgenic mice over