Progress in Neuropsychopharmacology & Biological Psychiatry 77 (2017) 23–31

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

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

Minocycline reduces inflammatory parameters in the brain structures and serum and reverses memory impairment caused by the administration of amyloid β (1-42) in mice

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ARTICLE INFO

Keywords:
Alzheimer's disease
Minocycline
Spatial memory
Neuroinflammation
Neurotrophins

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common type of age-related dementia. Cognitive decline, beta-amyloid (Aβ) accumulation, neurofibrillary tangles, and neuroinflammation are the main pathophysiological characteristics of AD. Minocycline is a tetracycline derivative with anti-inflammatory properties that has a neuroprotective effect. The aim of this study was to evaluate the effect of minocycline on memory, neurotrophins and neuroinflammation in an animal model of AD induced by the administration of Aβ (1-42) oligomer. Male BALB/c mice were treated with minocycline (50 mg/kg) via the oral route for a total of 17 days, 24 h after intracerebroventricular administration of Aβ (1-42) oligomer. At the end of this period, was performed the radial maze test, and 24 h after the last minocycline administration, serum was collected and the cortex and hippocampus were dissected for biochemical analysis. The administration of minocycline reversed the memory impairment caused by Aβ (1-42). In the hippocampus, minocycline reversed the increases in the levels of interleukin (IL-1β), Tumor Necrosis Factor- alpha (TNF-α) and, IL-10 caused by Aβ (1-42). In the cortex, AD-like model increase the levels of IL-1β, TNF-α and, IL-4. Minocycline treatment reversed this. In the serum, Aβ (1-42) increased the levels of IL-1β and IL-4, and minocycline was able to reverse this action, but not to reverse the decrease of IL-10 levels. Minocycline also reversed the increase in the levels of Brain-derived neurotrophic factor (BDNF) in the hippocampus caused by Aβ (1-42), and reduced Nerve Growth Factor (NGF) increases in the total cortex. Therefore, our results indicate that minocycline causes improvements in the spatial memory, and cytokine levels were correlated with this effect in the brain it. Besides this, minocycline reduced BDNF and NGF levels, highlighting the promising effects of minocycline in treating AD-like dementia.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder in which the main risk factor is age, with data showing a considerable increase in incidence rates after the age of 60 years. The chance of developing the disease increases as people get older (Dubois et al., 2016; Herrup, 2010; Savva et al., 2009). AD is the most common type of age-related dementia and is accompanied by memory loss and cognitive impairment (Ismail et al., 2011). AD causes visuospatial disorientation associated with cortical atrophy, which in turn leads to visuospatial memory impairments (Foxe et al., 2016; Mapstone et al., 2003). The Hippocampus and caudate nucleus are critical regions involved in spatial memory and navigation strategies (Craig and McBain, 2015; Dahmani and Bohbot, 2015). Atrophy of the hippocampus is a well-described feature of AD, and is directly associated with an increased risk towards the development and progression of AD (Apostolova et al., 2006; Chetelat et al., 2008; Mungas et al., 2005; Teipel et al., 2013).

AD initializes with a decline in episodic memory that is often mistaken for normal cognitive deficiencies due to aging, and is subsequently classified in the following stages: mild cognitive impair-

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http://dx.doi.org/10.1016/j.pnpbp.2017.03.010
Received 26 October 2016; Received in revised form 15 March 2017; Accepted 19 March 2017
Available online 21 March 2017

ment (MCI), early AD (EAD), and late-stage AD (LAD) (Butterfield et al., 2013). The disease is neurodegenerative and progressive, with different rates of progression being observed amongst AD patients (Zhao et al., 2014). However, in the last stage of the disease, the ultimate outcome is death. Presently, the only drug treatments approved for use with AD are forms of palliative care, acting to prevent any worsening of the patients' symptoms. There is currently no treatment leading to a cure of the disease (Ramirez-Bermudez, 2012).

The most described pathophysiological features of AD are the extracellular accumulation of beta-amyloid peptide (Aβ1-42) and neurofibrillary tangles (NFT) (Sery et al., 2013). The accumulation of Aβ1-42 peptide forms extracellular plaques called “senile plaques” that occur due to an increased and abnormal processing of the Aβ precursor protein (APP), which leads to an interruption of synapse function (Buoso et al., 2010). When α-secretase cleaved APP, it forms a nontoxic soluble extracellular fragment, sAPPα. When β-secretase cleaved APP, it generates a soluble extracellular fragment called sAPPβ. The latter γ-secretase forming Aβ 40-42 fragments, called Aβ or Aβ oligomers (Aβ aggregated form). It is toxic within the CNS, and can lead to neuroinflammation via the activation of the innate immune system (Heneka et al., 2014; Puzzo et al., 2015).

Neuroinflammation is an important pathophysiological feature of AD. It has recently attracted a number of studies, since it has been discovered that there are many polymorphisms within the genes related to the inflammatory responses, endocytosis and phagocytosis that have been observed in AD patients (Lambert et al., 2013). Neuroinflammation is involved in several neurodegenerative processes due to the production of inflammatory factors such as cytokines and chemokines (Tan et al., 2013). In addition, many changes occur in the levels of neurotrophins (Budni et al., 2015a). Increases in the levels of pro-inflammatory cytokines have been observed in the serum, brain and cerebrospinal fluid (CSF) of AD patients (Dursun et al., 2015; Jack et al., 2013; Zhang et al., 2013). Many studies have directly associated the levels of cytokines with the cognitive decline seen in AD patients at all stages, as it happens in older people without cognitive impairment or those suffering with mild cognitive impairment (Harries et al., 2012; Solfrizzi et al., 2006; Westin et al., 2012). However, there are no drugs approved for neuroinflammation in AD.

Minocycline is a tetracycline that possesses anti-inflammatory properties that are independent of its antimicrobial action. This drug is able to cross the blood brain barrier and presents a neuroprotective capacity (Kuang et al., 2009). In preclinical studies, minocycline has been shown to provide neuroprotective activity in various neurodegenerative diseases such as in Parkinson's disease (Wu et al., 2002), amyotrophic lateral sclerosis (Zhu et al., 2002), Huntington's disease (Chen et al., 2000) and AD, particularly within the transgenic mice used in some studies (Choi et al., 2007; Noble et al., 2009; Parachikova et al., 2010). Thus, some of the mechanisms of action for minocycline are already being studied (Budni et al., 2016). In addition, there is currently a phase II clinical trial taking place that is testing the effects of minocycline on AD patients (London, 2015). Therefore, the aim of this study was to evaluate the effects that minocycline has on memory and the levels of cytokines and neurotrophins in an AD-like animal model of dementia induced via the administration of amyloid β (1-42) oligomers.

2. Material and methods

2.1. Animals

Male BALB/c mice (100 day old, weighing 30–40 g) were used in this study. The animals were kept under standard conditions of a 12-h light/dark cycle, with food and water available ad libitum. They were housed in plastic cages with soft bedding. All manipulations were performed between 8:00 a.m. and 4:00 p.m. Local ethics committee (Ethics Committee on Animal Use – CEUA of the Universidade do Extremo Sul Catarinense) approved this study. ll experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals, as well as under the Brazilian Society for Neuroscience and Behavior recommendations for animal care.

2.2. Administration of Amyloid β (1-42) oligomer

The β-amyloid 1-42 peptide (Tocris Bioscience, Bristol, UK) was dissolved in a PBS solution (137 mM NaCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4; 2.7 mM KCl; pH 7.5) at a concentration of 1 μg/μL. It was then incubated for 7 days at 37 °C to allow the formation of birefringent fibril-like structures (oligomers Aβ) (Resende et al., 2008; Ruiz-Muñoz et al., 2011; Ueda et al., 1994). The solution was stored at − 20 °C until use, whereby it was diluted to the final dose of 400 pmol/site in artificial cerebrospinal fluid (ACSF) (24 mM NaCl; 2.5 mM KCl; 2.0 mM MgSO4; 1.25 mM KH2PO4; 26 mM NaHCO3; 10 mM glucose; 4 mM sucrose) before being used. The mice were anesthetized with isoflurane, and the Aβ oligomers were injected via microinjections using a Hamilton 10 μl syringe connected to a specially made 28-gauge stainless steel needle that was 3 mm in length. The needle was inserted unilaterally at a position 1 mm lateral to bregma, 1 mm posterior and 2.5 mm deep from the pial surface. The needle was inserted directly through the skin and skull and into the lateral ventricle, which was targeted by visualizing an equilateral triangle between the eyes and the center of the skull to locate the bregma (Gomes et al., 2013; Laursen and Belknap, 1986). The Aβ oligomers were injected in a volume of 4 μl over a period of 10s, followed by a 10 s delay to allow diffusion.

2.3. Drugs and treatment

Minocycline (Minocycline hydrochloride; Ranbaxy Laboratories, Madhya Pradesh, Índia) was diluted in water for administration at a dose of 50 mg/kg of body weight, using the oral route. It was administered by gavage, once a day, for a period of 17 days (Ferretti et al., 2012; Garwood et al., 2010). The treatment started 24 h after the injection of Aβ 1-42 oligomer. The animals were randomized into four groups: Control animals (receiving ACSF i.c.v. and water v.o.) or minocycline control (ACSF i.c.v. and minocycline 50 mg/kg v.o); and the AD-like animal model control (receiving Aβ 1-42 oligomer i.c.v. and water v.o) or AD-like model treated with minocycline (receiving Aβ 1-42 oligomer i.c.v. and minocycline 50 mg/kg v.o.). Testing using the radial arm-maze task started on the 14th day after Aβ 1-42 administration and ended on the 18th day, as explained below. On the day following the radial arm-maze task, which was twenty-four hours after the last administration of minocycline, serum was collected and the animals were then killed by decapitation. The dissected animals' cortex and hippocampus were frozen in liquid nitrogen and stored at − 80° for biochemical analysis.

2.4. Radial arm-maze task

On the 14th day after the administration of Aβ (1-42) oligomer, the animals were subjected to the radial arm-maze task. The radial arm-maze apparatus has 8-arms, which are numbered from 1 to 8 (48 × 12 cm), extending radially from a central area (32 cm diameter). The apparatus is placed 50 cm above the floor, and geometric shapes were positioned in the straight arms where the food was placed (visual cues). On the first day, each animal was placed in the apparatus for a total of 5 min, allowed only to explore, after returned to its cage. Prior to performing the maze task, the animals were kept on a restricted diet, and their body weight was maintained at 85% of their free-feeding weight over a period of one week, with only water being available ad libitum. On the first day of actual testing, the animals were placed in the apparatus, where food (cereal) had already been deposited in four of the eight arms, the food bearing arms having visual cues at the end of each arm. Over a period of 10 min, the entry into each arm (total errors