Neuroscience 284 (2015) 590–600

PROBUCOL MITIGATES STREPTOZOTOCIN-INDUCED COGNITIVE AND BIOCHEMICAL CHANGES IN MICE

D. B. SANTOS, * D. COLLE, E. L. G. MOREIRA, K. C. PERES, R. P. RIBEIRO, A. A. DOS SANTOS, J. DE OLIVEIRA, M. A. HORT, A. F. DE BEM AND M. FARINA *

Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

Abstract—Alzheimer's disease (AD) is a neurodegenerative disorder characterized by synaptic loss and cognitive impairments. Although AD is the most prevalent aging-related neurodegenerative disease, therapeutic strategies remain palliative. Recent studies have shown that probucol presents neuroprotective effects in experimental models of neurodegenerative disease. The present study aimed to investigate the potential protective effects of probucol against streptozotocin (STZ)-induced cognitive impairment and hippocampal biochemical changes (oxidative stress-related parameters, acetylcholinesterase (AChE) activity, cholesterol levels and β-secretase (BACE) protein levels) in mice. Adult Swiss mice received STZ [150 μg/bilateral, i.c.v.], and treated daily with probucol (≅10 mg/kg/day, in drinking water, for 5 weeks,). Twenty-one days after i.c.v. administrations, STZ-infused animals displayed significant deficits in cognition (evaluated in the displaced and new object recognition tasks), which were paralleled by a significant increase in hippocampal AChE activity. Moreover, STZ-infused mice showed increased levels of BACE and decreased glutathione reductase (GR) activity in the hippocampus compared with the control group. Probucol treatment significantly protected against the behavioral and hippocampal biochemical changes induced by STZ. However, it was unable to prevent STZ-induced increase of hippocampal BACE levels and did not change hippocampal cholesterol levels. It is noteworthy that probucol treatment increased the glutathione peroxidase (GPx) activity *per se* independent of STZ injection. The present findings are the first to show that i.c.v. STZ infusions are able to increase hippocampal BACE expression. Moreover, the results also show that probucol can counteract STZ-induced cognitive impairments and biochemical parameters independently of potential modulator effects toward BACE levels. The study is the first to report the protective effects of probucol against STZ-induced biochemical hippocampal changes and behavioral impairments, rendering this compound a promising molecule for further pharmacological studies on the search for therapeutic strategies to treat or prevent AD.

Key words: probucol, streptozotocin, cognitive impairment.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of progressive neurodegenerative dementia in the aged population, characterized by the deterioration of cognitive functions (Selkoe, 2001; Xie and He, 2011; Vassallo and Scerri, 2013). The pathogenesis of AD is complex and multifactorial, including inflammatory response, oxidative stress and aberrant cholesterol metabolism (Yamasaki et al., 2012). Of particular importance, the key pathological features in the AD brain are deposition of insoluble amyloid-β peptides (Aβ) and formation of neurofibrillary tangles that lead to neuronal cell death (Vardy et al., 2006). The processing of amyloid precursor protein (APP) by β-site APP-cleaving enzyme 1 (β-secretase; BACE-1) is the first and rate limiting step in releasing neurotoxic Aβ (Hills and Vacca, 2007). In this scenario, pharmacological strategies that decrease BACE-1 activity/expression have been proposed as potential therapeutic strategies to slow-down AD progression (Stamford and Strickland, 2013; Viayna et al., 2014; Viklund et al., 2014).

Oxidative stress, an imbalance between free radical production and the antioxidant systems, is well recognized for its contribution to the etiology of some conditions affecting neurodegenerative disorders, including AD (Butterfield, 2004; Zhu et al., 2005). This imbalance, which can be originated from an overproduction of free radicals or from a reduction in antioxidant defenses (Pereira et al., 2005), can affect all classes of macromolecules (lipids, proteins, and DNA), leading inevitably to neuronal dysfunction (Polidori and Mecocci, 2002). Brain cells are particularly vulnerable to oxidative

*Corresponding authors. Address: Departamento de Bioquímica, Centro de Ciências Biológicas, Campus Universitário, Trindade, Bloco C, Universidade Federal de Santa Catarina, CEP 88040-900 Florianópolis, Santa Catarina, Brazil. Tel: +55-4837212480; fax: +55-4837219672.

E-mail addresses: danubiabonfanti@hotmail.com (D. B. Santos), marcelo.farina@ufsc.br (M. Farina).

Abbreviations: AChE, acetylcholinesterase; aCSF, artificial cerebral spinal fluid; AD, Alzheimer's disease; APP, amyloid precursor protein; ANOVA, analysis of variance; BACE, β-secretase; CAT, catalase; CSF, cerebral spinal fluid; DTNB, 5,5′-Dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; GPx, glutathione peroxidase; GR, glutathione reductase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NADPH, β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate; NPSH, non-protein thiol; SOD, superoxide dismutase; STZ, streptozotocin.

http://dx.doi.org/10.1016/j.neuroscience.2014.10.019
0306-4522/

damage because of their high utilization of oxygen, low levels of antioxidant protection and the high polyunsaturated fatty acid content (Halliwell, 2001; Moreira et al., 2005). Particularly, oxidative damage has been reported to be associated to cognitive deficits, which represent a main symptom of dementia (Fukui et al., 2002). Of note, pro-oxidative events have been reported to positively modulate BACE activity (Tamagno et al., 2005), contributing to the neurodegenerative processes that take place in AD. Since oxidative damage is implicated in the etiology of neurological complications, treatment with antioxidants has been used as a therapeutic approach in various types of neurodegenerative diseases (Ansari et al., 2004; Ahmad et al., 2005).

In addition, probucol, a lipid-lowering agent, is a diphenolic compound with anti-inflammatory and antioxidant potential that reduces atherosclerosis and restenosis in coronary arteries (Yamashita and Matsuzawa, 2009). Furthermore, probucol is able to reverse the myocardial changes and increase the lifespan of the mice (Braun et al., 2003). Besides its lipid-lowering properties, a growing body of evidence shows that it also presents neuroprotective effects in experimental models (Farina et al., 2009; Colle et al., 2012). Our research group has continuously been investigating the pharmacological actions of the probucol manly in the central nervous system (Moreira et al., 2012; Santos et al., 2012; Colle et al., 2013; Ribeiro et al., 2013). Probucol ameliorated memory deficits in a mouse-based AD type experimental model due, in part, to its antioxidant potential (Santos et al., 2012; dos Santos et al., 2013). Interestingly, a recent study demonstrated that patients with mild-to-moderate AD that received probucol in cumulative doses showed a dose-dependent increase in apoE levels in the cerebral spinal fluid (CSF). Overall, this improvement in CSF apoE concentration in probucol-treated subjects was positively correlated with cognitive performance, decline in p-tau, and scavenging of total amyloid into the CSF (Poirier et al., 2014). Of note, this compound has been related as a strong candidate for the prevention and treatment of major disabling age-related neurodegenerative disorders (Champagne et al., 2003; Santos et al., 2012; Colle et al., 2013; Ribeiro et al., 2013).

Intracerebroventricular–streptozotocin (i.c.v., STZ) injection in rodents has provided a useful animal model of cognitive impairment. This condition is characterized by progressive deterioration of cognition, cerebral glucose and energy metabolism deficits, along with oxidative stress (Hoyer and Lannert, 1999; Ishrat et al., 2006; Hoyer and Lannert, 2008; Deshmukh et al., 2009). In this experimental model, STZ has been administered in subdiabetogenic doses in rodents and causes reduction on the cerebral energy metabolism, which triggers cognitive dysfunction by inhibiting the synthesis of adenosine triphosphate (ATP) and acetyl CoA. In addition, i.c.v.–STZ-treated animals present cholinergic deficiency supported by reduced choline acetyltransferase (ChAT) activity (Prickaerts et al., 1999; Ishrat et al., 2006) and increased acetylcholinesterase (AChE) activity in the hippocampus (Sonkusare et al., 2005; Ishrat et al., 2006). The enhancement of cholinergic activity by inhibition of AChE enzyme is the mainstay of symptomatic treatment of dementia (Levy et al., 1999; Ohnishi et al., 2013).

Nonetheless, still there is a lack concerning the specific mechanism of action of the probucol in the central nervous system (CNS). In this regard, the purpose of the present study investigated possible targets of its neuroprotective role against i.c.v. STZ injection in the hippocampus of mice. Behavior tests were performed and AChE activity, BACE levels and oxidative stress markers were evaluated in the mouse hippocampus in an attempt to elucidate mechanisms of neuroprotection.

EXPERIMENTAL PROCEDURES

Chemicals and antibodies

β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH), dimethyl sulfoxide, glutathione reductase (GR) from baker's yeast, reduced glutathione, epinephrine, hydrogen peroxide, *tert*-butyl hydroperoxide and probucol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Goat polyclonal antibody against C-terminal synthetic BACE peptide of human origin, mouse monoclonal anti-β-actin primary antibody and protein A/G horseradish peroxidase-conjugated secondary antibody were purchased from Santa Cruz (Santa Cruz, CA, USA). All other chemicals were of the highest grade available commercially.

Animals

Adult Swiss male mice (5-month-old), from our own breeding colony, were maintained at 21 ± 2 °C, on a 12-h light: 12-h dark cycle, with free access to food and water. All experiments were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology, adopted by the Society of Toxicology (1989) and were approved by ethics committee for animal use at the Universidade Federal de Santa Catarina (PP00546/CEUA 23080.037849/2010-71/UFSC).

Experimental protocol. To investigate the effects of probucol on an experimental model of cognitive damage, mice were randomly divided into 2 experimental groups (total n = 35). The animals were treated daily, for 5 weeks, with probucol (approximately 10 mg/kg/day, drinking water, *ad libitum*) (Siveski-Iliskovic et al., 1995; Colle et al., 2013) or vehicle (2% DMSO, drinking water, *ad libitum*). After 2 weeks of treatment, mice were re-divided into other two groups (a total of four experimental groups) and exposed to i.c.v. injection of STZ (STZ groups) or vehicle (control groups) twice (days 1 and 3, as shown in Fig. 1). Control groups received i.c.v. injection of artificial cerebral spinal fluid (aCSF- 147 mM NaCl; 2.9 mM KCl; 1.6 mM MgCl, 1.7 mM CaCl and 2.2 mM dextrose) (Agrawal et al., 2010). The STZ was administrated in the dose 75-μg/site bilateral (Weinstock et al., 2001).