Biomedicine & Pharmacotherapy 87 (2017) 135–144

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Riluzole ameliorates learning and memory deficits in Aβ25-35-induced rat model of Alzheimer's disease and is independent of cholinoceptor activation

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ARTICLE INFO

Article history:
Received 17 August 2016
Received in revised form 5 December 2016
Accepted 16 December 2016

Keywords:
Riluzole
Alzheimer's disease
$A\beta_{25-35}$
Learning and memory
Oxidative stress
Cholinergic receptor
Acetylcholinesterase

ABSTRACT

Alzheimer's disease (AD) is a major global public health concern and social care problem that is associated with learning, memory, and cognitive deficits. Riluzole is a glutamate modulator which has shown to improve memory performance in aged rats and may be of benefit in Alzheimer's disease. In the present study, its beneficial effect on attenuation of learning and memory deficits in $A\beta_{25-35}$-induced rat model of AD was assessed. Riluzole administration at a dose of 10 mg/kg/day *p.o.* improved spatial memory in Morris water maze and retention and recall in passive avoidance task and its protective effect was not neutralized following intracerebroventricular microinjection of muscarinic or nicotinic receptor antagonists. Further biochemical analysis showed that riluzole pretreatment of intrahippocampal Aβ-microinjected rats is able to attenuate hippocampal AChE activity and lower some oxidative stress markers, i.e. MDA and nitrite, with no significant change of the defensive enzyme catalase. Furthermore, riluzole prevented hippocampal CA1 neuronal loss and reduced 3-nitrotyrosine immunoreactivity. It is concluded that riluzole could exert a protective effect against memory decline induced by intrahippocampal $A\beta_{25-35}$ through anti-oxidative, anti-cholinesterase, and neuroprotective potential and its beneficial effect is possibly independent of cholinoceptor activation.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and is considered as the most prevalent form of dementia. The World Health Organization (WHO) reports that 47.5 million people were afflicted with dementia in March 2015 and 7.7 million new cases are reported each year due to accelerated population aging and a higher life expectancy [1]. The clinical symptoms of AD comprise a progressive decline in cognitive and behavioral performance, finally leading to memory deterioration, confusion, agitation, and difficulty in performing the daily activities. AD-associated disability and dependence impose a high social and economic burden on the health systems [2]. There is still no decisive cure for AD and currently used treatments are moderately effective in early stages of its pathologic process [2]. Although main pathologic hallmarks of AD focus on protein accumulation such as amyloid beta (Aβ) plaque and neurofibrillary tangles in the affected brain, however, other pathologies including enhanced inflammation and oxidative stress, cholinergic dysfunction, and synaptic atrophy are also observed [3]. A decrease in the number of nicotinic and muscarinic acetylcholine receptors as a result of severe degeneration of cholinergic neurons extending from the basal forebrain to the cortical and hippocampal areas are also acknowledged as one of the prominent features of AD [4,5]. In addition to degradation and dysfunction of cholinergic receptors, an inappropriate change in activity and level of acetylcholinesterase (AChE) is also strongly involved in cognitive deficits associated with AD and for this reason AChE inhibitors may be of benefit for management of mild to moderate AD [6]. Soluble oligomers of Aβ in the brain lead to neurodegeneration and impairment of synaptic function through interaction with glutamatergic signaling pathways [7,8]. Glutamate is responsible for most of excitatory neurotransmissions in

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http://dx.doi.org/10.1016/j.biopha.2016.12.067
0753-3322/

the central nervous system, especially in the cortical and hippocampal regions and although normal functioning of glutamate system is essential for regular neuronal functions such as learning and memory, however, excess glutamate stimulation is toxic and may lead to neuronal loss [9,10]. Furthermore, overactivation of ionotropic receptors, especially *N*-methyl-D-aspartate (NMDA) by high concentrations of glutamate could lead to excess Aβ production with the consequent oxidative stress-induced neurotoxicity; hence, blocking NMDA receptors and reducing presynaptic glutamate release can be important therapeutic strategies for AD [9].

Riluzole (2-amino-6-(trifluoromethoxy) benzothiazole) is a glutamate release inhibitor [11] and a neuroprotective agent and is the only drug approved for treatment of amyotrophic lateral sclerosis [11,12]. Research studies have assessed and suggested the use of riluzole for the treatment of neurodegenerative diseases such as Parkinson's disease [13] and mood and anxiety disorders in humans or animal models [14,15]. The mechanism of action of riluzole is not fully understood. Inhibition of voltage-gated sodium channels, followed by decreased presynaptic glutamate release and elevated astrocytic uptake of extracellular glutamate are suggested mechanisms [16,17]. Riluzole has been observed to exert an antioxidative effect, perhaps by decreasing lipid peroxidation and inhibiting cytosolic phospholipase A2 [18] and is able to attenuate inflammation in a preclinical rodent model of cervical spinal cord injury [19]. Riluzole could also attenuate memory impairment linked to aging [20]. Recently, a study by Hunsberger et al. in 2015 has suggested riluzole as an applicable therapeutic approach to regulate glutamate in vulnerable circuits for those at risk for developing AD [21]. However, the beneficial effect of riluzole has not been investigated on $A\beta_{25-35}$-induced rat model of AD. Therefore, we designed this study to examine the effect of oral administration of riluzole on amelioration of learning and memory deficits in $A\beta_{25-35}$-induced rat model of Alzheimer's disease with emphasis on possible involvement of cholinergic pathway.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (250–300 g, purchased from Iran University of Medical Sciences animal breeding facility, Tehran, Iran) were housed in standard cages under 12 h light/dark cycle (lights on at 7 a.m.), at a temperature range of 21–23 °C and a humidity of 40–50% with free access to food and water throughout the study. The rats were kept in this environment for at least 10 days prior to the experiments. This study was conducted according to guidelines for the care and use of laboratory animals stipulated by National Institutes of Health (NIH) and approved by Ethics and Research Committee of Iran University of Medical Sciences (Tehran, Iran) in 2014.

2.2. Experimental design

The rats (n = 64) were randomly allocated to the following equal-sized groups: sham, riluzole-treated sham (Sham + Riluzole), Aβ, riluzole-treated Aβ (Aβ + Riluzole), riluzole-treated Aβ receiving atropine as a muscarinic receptor antagonist (Aβ + Riluzole + Atropine), and riluzole-treated Aβ receiving mecamylamine as a nicotinic receptor antagonist (Aβ + Riluzole + Mecamylamine). To induce AD model in the rats, they were first anesthetized with a combination of ketamine (Ratiopharm, Germany; 100 mg/kg, i.p.) and xylazine (Ratiopharm, Germany; 5 mg/kg, i.p.) and then placed and fixed in a stereotaxic apparatus (Stoelting, USA) with incisor bar 3.3 mm below the horizontal plane and ear bars in a symmetrical position. The scalp was cleaned with an iodine solution and after a midline sagittal incision, the CA1 area of the hippocampus was targeted at 3.5 mm posterior to the bregma, ±2 mm lateral to the sagittal suture, and 2.8 mm below the dura, in accordance to the stereotaxic atlas [22] and with appropriate adjustments. After drilling the marked points on the skull, 2 μl of sterile normal saline solution containing 10 μg of aggregated Aβ (25–35) (5 μg/μl; Sigmaaldrich, USA) were injected bilaterally into the CA1 of the dorsal hippocampus [23]. To produce a higher toxicity for Aβ, saline-dissolved $A\beta_{25-35}$ was incubated at 37 °C for 72 h to allow aggregation and fibril formation [23]. Riluzole (Sigmaaldrich, USA) was dissolved in 30% Cremophor (Sigmaaldrich, USA) and was administered *p.o.* (by a rodent gavage needle) at a dose of 10 mg/kg/day for 10 days till 1 h pre-surgery. Riluzole dose was selected based on experimental studies on its neuroprotective effect in early phase Parkinson's disease model induced in the marmoset by 1-methyl-1,2,3,6-tetrahydropyridine (MPTP) [24] and its protective effect on MPTP-induced depletion of dopamine and its metabolites in mouse brain [25]. The groups Aβ +Riluzole + Atropine and Aβ + Riluzole + Mecamylamine received atropine (5 μl at a concentration of 1 μg/μl dissolved in DMSO and diluted in aCSF and microinjected i.*c.v.*) and mecamylamine-HCl (5 μl at a concentration of 5 μg/μl dissolved in DMSO and diluted in aCSF and microinjected i.*c.v.*), respectively, 30 min before the surgery. The dose of cholinergic receptor antagonists was chosen form an earlier study [26]. The aCSF solution contained 120 mM NaCl, 1.15 mM CaCl2, 3 mM KCl, 0.8 mM MgCl2, 27 mM NaHCO3, and 0.33 mM NaH2PO4 and pH was adjusted to 7.2. The *i.c.v.* coordinates were 1.4 mm lateral and −1 mm posterior to bregma

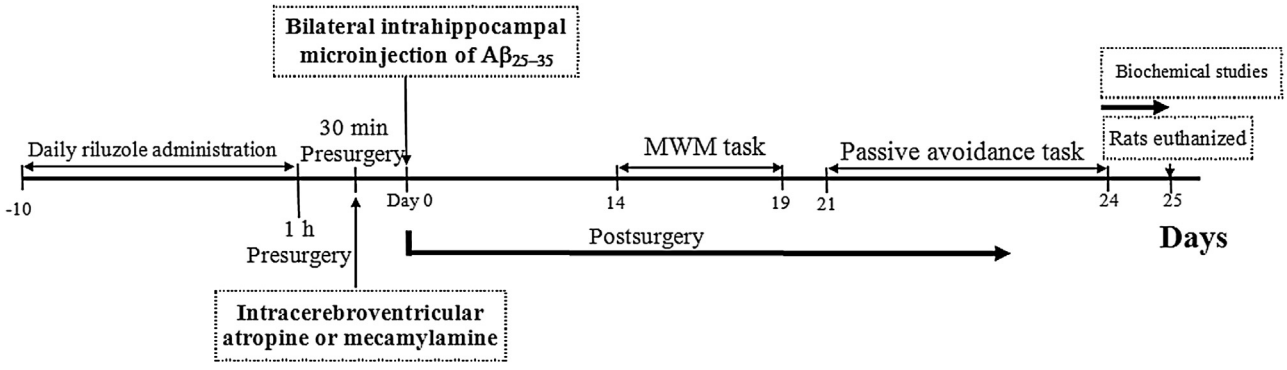

Fig. 1. Experimental protocol for treatments and behavioral tests. For induction of AD, 10 μg of aggregated Aβ25-35 was injected bilaterally into the CA1 area of the dorsal hippocampus. Riluzole was administered *p.o.* at a dose of 10 mg/kg/day for 10 days till 1 h pre-surgery. The groups Aβ + Riluzole + Atropine and Aβ + Riluzole + Mecamylamine received atropine (i.c.v.) and mecamylamine-HCl (i.c.v.) 30 min before the surgery.