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The beneficial effects of riluzole on GFAP and iNOS expression in intrahippocampal Aβ rat model of Alzheimer's disease

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ABSTRACT

Background and Objective: Alzheimer's disease (AD) is a neurodegenerative disorder specified by deposition of β-amyloid (Aβ) and neuronal loss that leads to learning and memory disturbances. One of the most important causes of AD is glutamate-dependent excitotoxicity in brain regions that is vulnerable to AD. According to previous reported results, it was revealed that riluzole, as a glutamate release inhibitor, could improve learning and memory in an experimental model of AD. The aim of this study was to determine the effects of riluzole on Hippocampal astrogliosis and amyloidosis in a rat model of AD.

Materials and Methods: In the present study, the effects of riluzole administration at a dose of 10 mg/kg/day *p.o.* on hippocampal glial fibrillary acid protein (GFAP) as an astrogliosis marker and inducible nitric oxide synthase (iNOS) level in Aβ (25-35)-injected rats was evaluated.

Results: The results showed that in Aβ (25–35)-injected rats, the intrahippocampal GFAP ($p<0.05$) and iNOS ($p<0.0001$) level increased as compared to sham group. Administration of riluzole to Aβ (25–35)-injected rats could significantly decrease iNOS level ($p<0.05$) and had no effect on GFAP level.

Conclusion: This study indicates that in rat model of AD, riluzole is able to attenuate NO synthesis with reducing hippocampal iNOS level, probably through inhibition of glutamatergic signaling pathway.

1. Introduction

Alzheimer's disease (AD) is the most frequent type of dementia that causes memory loss and cognitive impairment in older people (1). It has been predicted that total number of people with dementia in Europe will be about 14 million in 2050 that 80% of those dementia cases result from AD (2). Common form of Alzheimer's disease is sporadic and people older than 60 years are at risk with this disease (3). In addition to dementia, two specified pathological parameters including intraneuronal neurofibrillary tangles comprised of hyperphosphorylated tau protein and senile plaques composed of amyloid-β (Aβ) peptide are seen at cortical and subcortical regions of brain in AD patients (4). The available evidence suggests that accumulation of Aβ can directly leads to hyperphosphorylation of tau protein, oxidative stress, synaptic plasticity and transmission deficiency, and neuron loss (5, 6).

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Since Aβ is able to bind to excitatory synaptic receptors on dendritic spines and decreases its numbers (7), it exerts its non-beneficial effects on synaptic plasticity through blocking long-term potentiation (LTP) (8) and evoking long-term depression (LTD) (5). The main excitatory neurotransmitter in central nervous system is glutamate. The glutamatergic NMDA receptors that have major role in regulating calcium homeostasis, affect deeply learning and memory. Dysfunction of these receptors as seen in AD, causes excitotoxicity and neuronal loss (9).

Inflammation is also associated with neuronal death in AD (10). Some inflammatory agents including lipopolysaccharide (LPS) and cytokines increase the NO production through stimulation of iNOS expression in microglia, astrocytes and neurons (11). Huge amount of NO can inhibit the mitochondrial cytochrome oxidase activity that leads to neuronal loss (12). On the other hand, deficiency in mitochondrial respiration depolarizes the neurons and in turn induces glutamate release and NMDA receptors stimulation (13).

Riluzole as treatment drug for amyotrophic lateral sclerosis activates small-conductance calcium-activated potassium channels (14). In addition, riluzole inhibits glutamatergic signaling pathway and increases the scavenging of glutamate (15). Regarding to inhibitory effect of riluzole on glutamate release, this study was designed to investigate the possible protective effect of riluzole against hippocampal GFAP and iNOS levels in beta amyloid-induced rat model of Alzheimer's disease.

2. Materials and Methods

2.1. Animals

This study was carried out on adult male Wister rats (local animal house, 250-300 g). They were kept four per cage in a colony room with temperature and light/dark cycle-controlled. The animals had free access to water and food. This study was conducted in accordance with the policies stipulated in the Guide for the Care and Use of Laboratory Animals (NIH) and by the Research Council of Iran University of Medical Sciences (Tehran, Iran).

2.2. Experimental procedure

Rats (n= 37) were randomly allocated to the following equal groups: (1) sham operating (Sh); (2) Riluzole-sham operating (Sh+R), Aβ injection (A-beta); (3) Riluzol-treated Aβ injection (A-beta + R). For stereotaxic surgery, rats were anesthetized with a combination of ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) and then placed in a Stoelting stereotaxic apparatus(incisor bar -3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution and incised on the midline, and a burr hole was drilled through the skull and $A\beta_{(25-35)}$ was injected at coordinates of -3.5 mm posterior to bregma, 2 mm lateral to sagittal suture, and 2.8 mm below dura, according to the stereotaxic atlas. The dosage was chosen according to the results of our pilot study. Animals in the Aβ group were bilaterally injected in the dorsal hippocampus with 4 µl of a solution containing $A\beta_{(25-35)}$ (2 nmol/4 µl; Sigma, USA). The amount of Aβ (0.5 nM/µl dissolved in 0.9% normal saline; pH=8.0) was chosen based on our earlier experiment, and the solution was prepared according to a previously described protocol (16) and then immediately stored at 70 °C until used. Sham-operated rats received 4 µl of 0.9% normal saline instead of Aβ solution. Riluzole (Sigma, USA) was dissolved in 30% Cremophor (Sigma, 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 a previous study (17).

2.3. Assessment of hippocampal glial fibrillary acidic protein (GFAP) and inducible nitric oxide synthase (iNOS)

In each group (n=5), after providing hippocampal supernatant, the GFAP and iNOS level was evaluated by enzyme-linked immunosorbent assay and commercial kits. The sample absorbance was read at 450 nm by Synergy HT microplate reader (BioTek, Winooski, Vermont,USA) and values were expressed as ng/ml of supernatant.

2.4. Statistical analysis

Data are presented as Mean±SEM. One-was ANOVA and Tukey *post-hoc* tests were used for analysis of data with significance level set at 0.05.

3. Results

Hippocampal GFAP and iNOS

As shown in Figures 1 and 2, GFAP (P<0.05) and iNOS (P<0.01) levels were significantly increased in A-beta group as compared to sham group. Administration of riluzole had no effect on GFAP level, however, it significantly decreased iNOS level in A-beta group (P<0.05).

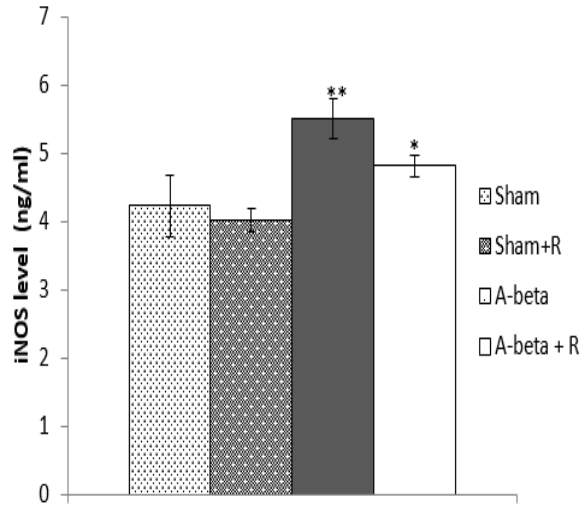

Fig. 1. Hippocampal inducible nitric oxide synthase (iNOS)

**P < 0.01(Versus Sham), *P < 0.05 (Versus Ab). Values are means ± SEM.

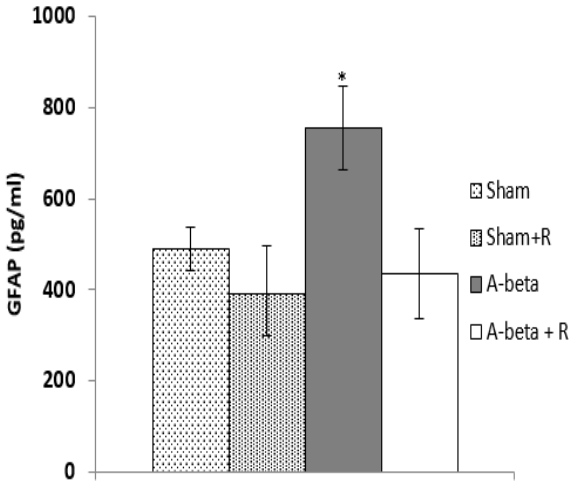

Fig. 2. Hippocampal glial fibrillary acidic protein (GFAP) *P < 0.05 as compared to Ab group. Values are means ± SEM.

Discussion

This study was designed to investigate the effect of riluzole on amyloidosis and GFAP and iNOS expression in an intrahippocampal $A\beta_{(25-35)}$-injected rat model of AD. The major findings were as follows: two weeks post-surgery, the $A\beta_{(25-35)}$ -injected rats showed that GFAP and iNOS levels increases in the $A\beta_{(25-35)}$ -injected rats and pre-treatment of $A\beta_{(25-35)}$ -injected rats with riluzole significantly decreased the levels of iNOS.

NO has a noxious effect on cells through producing ROS and RNS. The three isoforms of nitric oxide synthase (NOS), neuronal, inducible and endothelial, is responsible for NO synthesis (18). In hippocampus, NO regulates synaptic plasticity (19). Some studies showed that hippocampal and intracerebroventricular administration of $A\beta_{(25-35)}$ intensified the nNOS expression into the cell (20). In addition, hippocampus exposure to $A\beta_{(1-40)}$ caused an increase in the expression of iNOS in glial cells and neurons (21). Rising of iNOS expression in hippocampus after exposure to Aβ is consistent with our results. iNOS expression has a direct effect on neurodegeneration in AD (22). Pro-inflammatory cytokines lead to microglia activation with its increased iNOS expression (23). Also, in brain cell culture, neuronal loss is induced by activation of iNOS and NMDA receptors (24). On the other hand, damage to nervous system causes to increase the number of astrocytes leading to reactive astrogliosis (25). Astrocytes exert their protective effects through reducing extracellular plaques of Aβ (26). It is observed that in animal model of alzheimer,s disease, infusion of $A\beta_{(1-40)}$ into rat brain increases the astroglial GFAP expression (27). In present study, GFAP reactivity was also increased after administration of $A\beta_{(25-35)}$.

As mentioned before, in Alzheimer's disease, neocortical and hippocampal neuronal death exerts some behavioral disturbances (28). Although glutamate is a main excitatory neurotransmitter, but a large body of evidence has shown that dysfunction of glutamatergic signaling pathways leads to cognitive, learning and memory disorders (29). Over-excitation of glutamate receptors causes neuronal death in AD (30). Deficiency in metabolism and insufficient energy production in neurons of AD patients

cause cell depolarization and excessive release of glutamate that this in turn leads to accumulation of Na^{+} and Ca^{++} into the neurons. The increase of intracellular Na^{+} and Ca^{++} has a key role in neuronal death. Aβ plaques make the neurons more susceptible to glutamate (31).

It seems that reduced glutamate release can have beneficial effects in preventing of destructive effects resulted from the accumulation of beta-amyloid. In vitro and in vivo studies have shown that riluzol has inhibitory effect on glutamate release through blocking voltage-dependent Na^{+} channels and change of presynaptic Ca^{++} dynamic (32). Also, riluzole increases scavenging ability of glutamate transporters, so that it increases the expression level of EAAT2 excitatory amino acid transporter2 in hippocampal neurons (33). In addition, as studies have shown, the total levels of tau are decreased after riluzole-treatment (34). On the other hand, riluzole exerts antioxidant and anti-inflammatory effects (35) and attenuates cognitive deficiency in animal model of AD (36). In our study, administration of riluzole, as a glutamate release inhibitor to rats receiving Aβ alleviated the astroglial GFAP immunoreactivity and iNOS production. Therefore, it seems that riluzole exerts a part of its neuroprotective effects on neurodegenerative diseases including Alzheimer's disease through interference in glutamate dynamic.

In conclusion, this study indicates that in rat model of AD, riluzole is able to attenuate NO synthesis with reducing hippocampal iNOS level, probably through inhibition of glutamatergic signaling pathway.

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