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Protective Effects of Troxerutin on β-Amyloid (1-42)-Induced Impairments of Spatial Learning and Memory in Rats

S. Babri[1], M. Amani[1,2], G. Mohaddes[3], A. Alihemmati[2], and H. Ebrahimi[2]

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In our study, we examined the protective effect of troxerutin, a natural bioflavonoid, on β-amyloid, Aβ (1-42)-induced impairments of learning and memory. Male Wistar rats (300-350 g) were divided into six groups, sham, Aβ (1-42)-, Aβ (42-1)-injected, and three groups Aβ (1-42)-injected plus treatment with three different doses of troxerutin (50, 150, and 300 mg/kg) for 8 consecutive days. The Aβ peptides were injected intracerebroventricularly (i.c.v.) into the right lateral ventricle; 8 days later, the Morris water maze test was performed to assess spatial learning and memory. Our results showed that troxerutin at lower doses (50 and 150 mg/kg) exerted no significant effect on Aβ-induced impairment of memory performance, but the dose of 300 mg/kg significantly protected against the above impairment of spatial learning and memory in Aβ (1-42)-treated animals. The beneficial effects of troxerutin may partly be due to its antioxidant and anti-inflammatory activities and to modifications in the cholinergic system.

Keywords: troxerutin, spatial learning and memory, Morris water mase, β-amyloid, Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a frequent cause of dementia in elderly peoples; it is accompanied by progressive cognitive decline and memory loss. Pathologic hallmarks of this disease include the formation of senile plaques, neurofibrillary tangles (NFTs), synapse loss, and neuronal dysfunction in different sites of the brain, in particular in the hippocampus [1-3]. Accumulation of amyloid β protein, Aβ, a main component of the senile plaques, in the brain initiates a cascade of events that ultimately lead to neuronal dysfunction and cognitive deficits. Other proposed mechanisms for AD include impairment of cholinergic transmission, oxidative stress, action of inflammatory agents, and glutamate-mediated excitotoxicity [4-6].

Troxerutin has been recognized as a trihydroxyethylated derivative of the natural bioflavonoid rutin; it has been found in tea, coffee, cereal grains, and a variety of fruits and vegetables. Troxerutin possesses a number of biological activities, such as anti-oxidative, anti-inflammatory, and nephroprotective, and is considered an effective agent in the treatment of cardiovascular diseases [7-9]. It was also demonstrated that troxerutin enhances the expression of nicotinic acetylcholine receptors and inhibits cholinesterase (AChE) activity in the brain [10].

Our earlier studies demonstrated that troxerutin alleviates Aβ-induced impairment of hippocampal long-term potentiation (LTP), an important mechanism involved in learning and memory, and reduces the amount of oxidative stress markers in the hippocampus of Aβ-treated rats (unpublished). According to these findings, we hypothesized that if some probable pathologic mechanisms involved in neurodegenerative diseases are suppressed by troxerutin, it may also manifest some protective action against AD. Therefore, in our behavioral study, we examined for the first time a protective effect of troxerutin on spatial learning and memory deficits in the Morris water maze (MWM) test in an Aβ-inducted rat model of AD.

METHODS

Animals and Treatments. Adult male Wistar rats (body mass 300-350 g) were obtained from the Pasteur Institute of Iran. They were maintained at an

[1–3] Tabriz University of Medical Sciences, Tabriz, Iran.
([1] Drug Applied Research Center, [2] School of Medicine, and [3] Neurosciences Research Center).
Correspondence should be addressed to M. Amani (e-mail: mohammad.amani@gmail.com).

ambient temperature of 22-24°C under a 12-h light-dark cycle, with lights off at 7.00 p.m. Food and water were provided *ad libitum* except during the behavioral tests. The behavioral tests were carried out between 10 a.m. and 4 p.m. All stages of the study were performed using protocols approved by the Research and Ethics Committee of the Drug Applied Research Center and were conducted under the recommended conditions of the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

The Aβ (1-42) peptide fragment and a nonamyloidogenic reverse peptide, Aβ (42-1, the control for Aβ (1-42) were purchased from Bachem (Switzerland); troxerutin was acquired from Merck (Germany), and Congo red and poly L-lysine solution were obtained from Sigma (USA). Aβ (1-42) and Aβ (42-1) were dissolved in sterile double-distilled water at a concentration of 2.25 mg/ml (according to the manufacturer's instructions), and 2.0 nmol of these agents in 4.0 µl was administered intracerebroventricularly (i.c.v.), as previously described [11]. To obtain the aggregated form, the peptide solutions were placed in an incubator at 37°C for 72 h. Troxerutin was dissolved in distilled water.

Animals were randomly divided into six groups (n = 10 in each group). These were sham, Aβ (1-42), Aβ (42-1), and Aβ (1-42) plus three doses of troxerutin (50, 150, and 300 mg/kg daily by gavage for eight consecutive days). The first treatment by the drug or vehicle was performed 1 h before i.c.v. Aβ or solvent microinjections.

Surgical Procedures. The rats were anesthetized i.p. with chloral hydrate (350 mg/kg) and placed in a Stoelting stereotaxic instrument (Stoelting Co., USA). The scalp was incised and retracted, and a small hole was drilled at an appropriate location in the skull to allow the insertion of a stainless steel injection cannula. The peptides or sterile double-distilled water were then injected through the cannula into the right lateral ventricle (AP, −0.8, ML, 1.6, and DV, 3.5 mm below the dura) during 5 min by a Hamilton microsyringe, and the needle was left in place for 5 min before it was slowly withdrawn. Coordinates were chosen based on the Paxinos and Watson rat brain atlas [12].

Morris Water Maze (MWM). The experimental apparatus consisted of a black circular pool (diameter 130 cm and height 60 cm) filled with water (23 ± 2°C) to a depth of 40 cm. The maze was divided into four equal quadrants, and release points in the quadrants were designed as N, E, S, and W. A hidden Plexiglas platform (10 × 10 cm) was placed in the center of the NW quadrant (submerged 1.5 cm below the water surface). Various prominent visual cues were present around the maze. A tracking system was used to measure the escape latency, path length, and swimming speed**.**

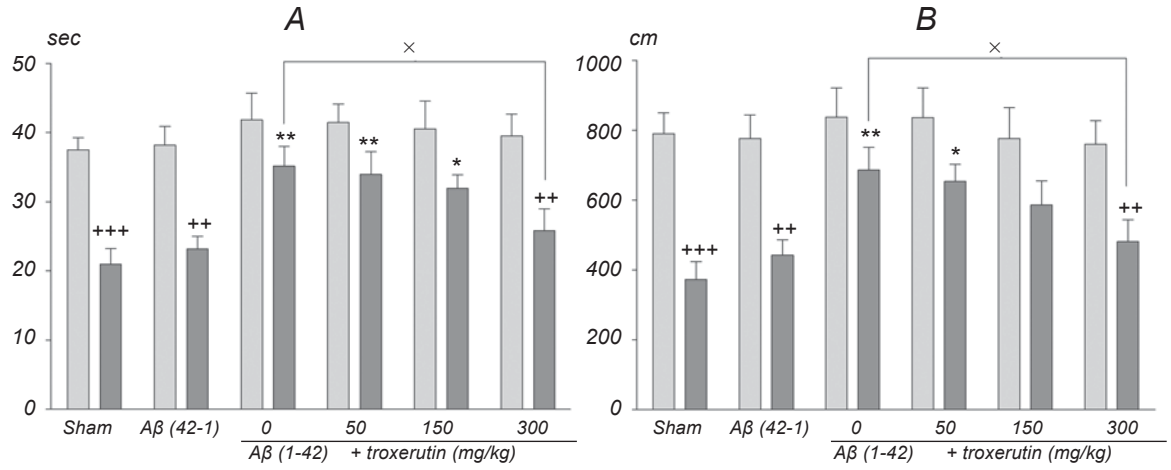
Eight days after surgery, the rats were trained in the water maze. The training session was performed in two consecutive blocks, and each block consisted of four trials with different starting positions that were similarly equally distributed between the first block and the last one [13]. Rats were left in the tank facing the wall and allowed to swim freely to the escape platform. If the animal failed to find the platform during 60 sec, it was gently guided to it. The animal was allowed to remain there for 20 sec and then was placed in a holding cage for a 30-sec-long period until the start of the next trial. The time interval until reaching the platform and the traveled distance were measured. At the end of the training sessions, the animals were returned to their home cages until a retention testing 24 h later. The probe trial consisted of a 60-sec-long free-swim period without a platform, the time spent in the target quadrant was recorded, and the percentage of the total time was calculated.

Histology. On completion of behavioral training, rats were perfused transcardially with a cold 10% formalin solution; the brain was removed and post-fixed in the same formalin solution for 48 h at room temperature. Subsequently, the brains were sectioned for evaluating the injection site.

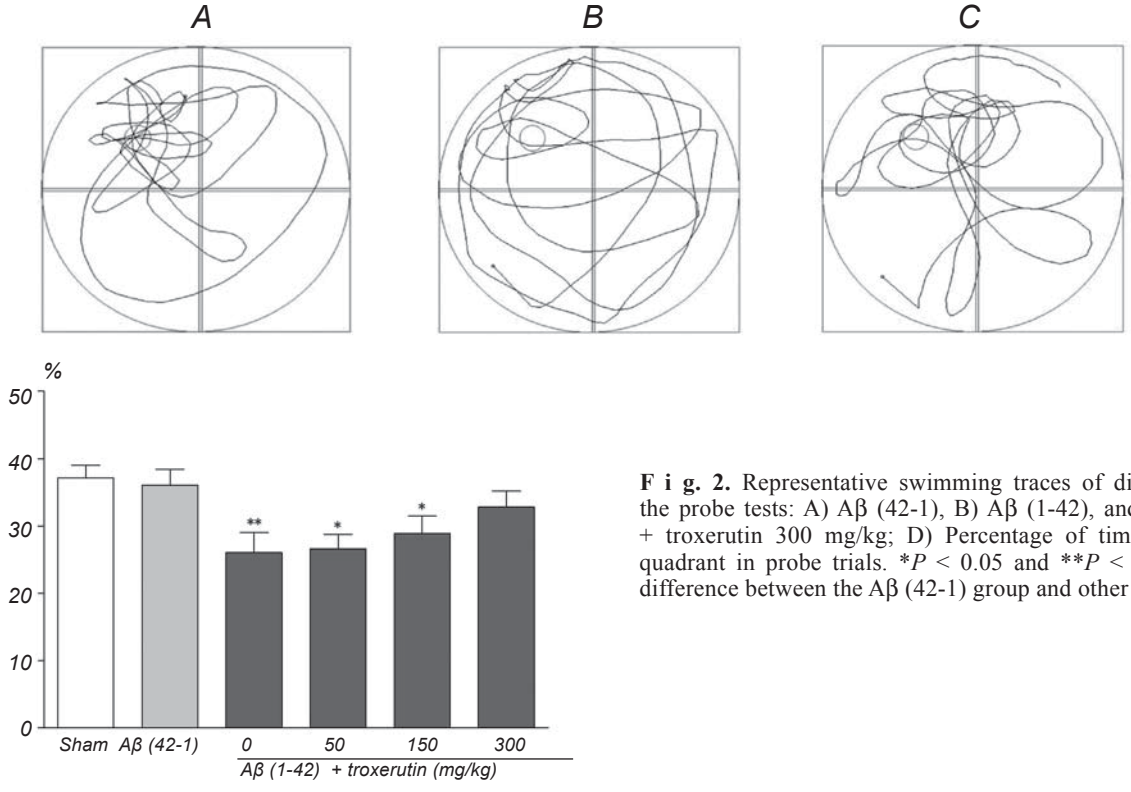
Statistical Analysis. All analyses were performed using IBM SPSS Statistics version 20. The statistical analysis of the data was carried out by one-way ANOVA followed by the LSD post-hoc test. The paired Student's t-test was used in each group for comparison between blocks 1 and 2, and the unpaired t-test was performed for comparison between the sham and Aβ (42-1) groups. A P value of less than 0.05 was considered to be statistically significant. Data are expressed as means ± s.e.m. for each group.

RESULTS

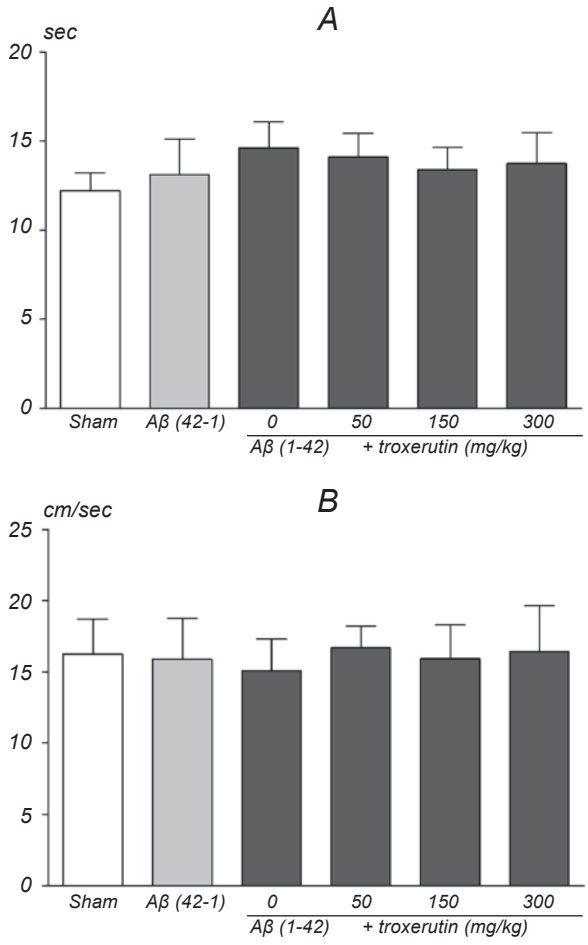
Intracerebroventricular injections of the reverse peptide Aβ (42-1) exerted no significant effect on rats in the MWM performance. The Student t-test showed no significant difference during acquisition



F i g. 1. Escape times (A) and distances traveled to find the hidden platform (B). Each block represents the average of four consecutive trials. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate the cases of significant difference between each block and its respective block in group Aβ (42-1) . $^{++}P < 0.01$, and $^{+++}P < 0.001$ indicate the difference between two blocks in each group. $^{x}P < 0.05$ indicates the difference between the group with troxerutin at a dose of 300 mg/kg and group Aβ (1-42) in the second block.



F i g. 2. Representative swimming traces of different groups in the probe tests: A) Aβ (42-1), B) Aβ (1-42), and C) Aβ (1-42) + + troxerutin 300 mg/kg; D) Percentage of time spent in target quadrant in probe trials. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate the difference between the Aβ (42-1) group and other groups.



F i g. 3. Escape times to find the visible platform (sec, A) and the swimming speed in the visible platform test (cm/sec, B). There was no significant difference in the escape times and swimming speed between different groups.

or retrieval in the Aβ (42-1) group in comparison with the sham group. The paired *t*-test for the escape time revealed a significant difference between two blocks in each group in both sham ($P < 0.001$) and Aβ (42-1) groups ($P < 0.01$). Administration of Aβ (1-42) noticeably impaired water maze learning in subsequent blocks in this group. Treatment with troxerutin at doses of 50 and 150 mg/kg exerted insignificant effects on this water maze acquisition impairment. Animals administered a dose of 300 mg/kg of troxerutin demonstrated improved spatial learning in Aβ (1-42)-treated rats, as the interval reaching the platform in the second block was significantly shorter than that in the first one ($P < < 0.01$) and there was a significant decrease in the escape time in this block relative to the respective block in the Aβ (1-42) group ($F(4,45) = 3.89$, $P < < 0.05$) (Fig. 1A).

Values of the traveled distance are shown in Fig. 1B. One-way ANOVA revealed a significant difference in the traveled distance to find the platform between the second block in the Aβ (1-42) group, as compared with the respective block in the Aβ (42-1) group ($F(4,45) = 3.26$, $P < 0.01$). Animals that received 50 mg/kg of troxerutin also showed a longer traveled distance in the second block relative to the respective block in the Aβ (42-1) group ($F(4,45) = 3.26$, $P < 0.05$). At the same time, administration of troxerutin at higher doses (150 or 300 mg/kg) decreased the traveled distance. There were insignificant differences between these groups in comparison with the Aβ (42-1) group, whereas this distance was significantly shorter in 300 mg/kg troxerutin-treated rats relative to the respective block in the Aβ (1-42) group ($F(4,45) = = 3.26$, $P < 0.05$) (B). One-way ANOVA performed for the test results showed the existence of a significant difference between the Aβ (1-42) group *vs* its control, the Aβ (42-1) group. Animals treated with Aβ (1-42) spent significantly lesser time in the target quadrant than the Aβ (42-1) group did ($F(4,45) = 2.89$, $P < < 0.01$), indicating noticeable memory impairment in this group. Administration of troxerutin in doses of 50 and 150 mg/kg did not exert a significant enhancing effect on this index. Whereas animals receiving 300 mg/kg troxerutin spent more time in the target quadrant, there was no significant difference between this group compared with the Aβ (42-1) group, indicating that memory improvement in this group is obvious (Fig. 2D).

Our findings demonstrated that there were no significant differences in the escape time to find the visible platform and in the swimming speed (Fig. 3). The results of this study suggest that i.c.v. injection of Aβ (1-42) had no effect on motivation, swimming speed, and motor function of animals, and impairment in the water maze task in Aβ-treated rats is due to cognitive deficits.

DISCUSSION

In our study, we investigated the protective effects of troxerutin in rats subjected to i.c.v. injections of Aβ (1-42); this is widely known as an animal

model of AD disease. Even a single microinjection of a nanomolar dose of Aβ (1-42) into the lateral ventricle effectively impaired learning and memory in the water maze. This result is consistent with the results of previous studies where Aβ (1-42) was also injected into the brain ventricle [14-16].

The hippocampus and associated areas of the temporal cortex are important for learning and play a critical role in the early stage of memory formation [17, 18]. The deposition of Aβ first occurs in these regions, especially in the hippocampus [19, 20].

In our study, oral administration of troxerutin, a derivative of the bioflavonoid rutin, at a dose of 300 mg/kg for 8 days in Aβ (1-42)-treated animals effectively protected against Aβ-induced impairment of learning and memory, as the escape time and traveled distance to find the platform in training tests and the time spent in the target quadrant in the probe test did not differ significantly from those in Aβ (42-1)-treated rats.

Except for a significant effect at a dose of 150 mg/kg of troxerutin on the traveled distance to find the platform, administration of troxerutin in doses of 50 and 150 mg/kg exerted no significant influence on Aβ-induced cognitive deficit; presumably, these doses were not enough to have such protective effects. Aβ (1-42) is a major constituent of the pathologic hallmark of AD, the so-called senile plaques. Previous studies have shown that Aβ-induced oxidative stress plays a pivotal role in the pathogenesis of disease that precedes neurodegenerative lesions in AD. In addition, neuroinflammatory responses may be observed in the brain due to early accumulation of Aβ; these events are associated with AD neuropathology [21, 22]. Troxerutin is well known as a potent antioxidant and anti-inflammatory agent [7, 23]. The protective effect of troxerutin on Aβ-induced impairment of spatial learning and memory might be ascribed, at least in part, to its antioxidant and anti-inflammatory effects. Strong evidence was obtained that Aβ peptides can produce cholinergic deficits, which is the main neuropathological feature of patients with AD [24, 25]. The density of presynaptic β7 nicotinic acetylcholine receptors (β7nAChRs), which are essential for learning and memory, was found to decrease in AD. Aβ binds to these receptors, decreasing the release of acetylcholine (ACh) and impairing the maintenance of long-term potentiation (LTP) as the main mechanism of learning and memory [26-28]. Aβ also negatively alters the muscarinic acetylcholine receptors, ACh synthesis, and ACh release [24, 29, 30].

Results of previous studies demonstrated that troxerutin enhances β7nAChR expression and decreases acetylcholinesterase (AChE) activity in the hippocampus, basal forebrain, and frontal cortex in D galactose-treated mice [10]. Accordingly, the protective effect of troxerutin in this study may partly be due to reversal of the changes occurring in the cholinergic system, such as increases in the level of β7nAChRs and amount of available ACh in Aβ-treated rats. Previous studies also demonstrated that advanced glycation end products (AGEs) are involved in pathologic changes in AD and other neurodegenerative disorders [31, 32]. AGEs have been identified in both senile plaques and neurofibrillary tangles (NFTs) in these diseases. Glycation of Aβ can promote aggregation of this peptide and formation of plaques and glycation of τ proteins; in addition, its hyperphosphorylation can enhance the formation of NFTs [33, 34]. It has been shown that administration of troxerutin decreases the AGE level in the hippocampus, basal forebrain, and frontal cortex [10]. On the other hand, excessive production of reactive oxygen species (ROSs), especially superoxide ions and hydrogen peroxide, is one of the main mechanisms of AGE-induced damage [35]. According to these findings, the beneficial effects of troxerutin appear to be partly due to its antioxidant and free radical-scavenging activities. Moreover, we demonstrated in our earlier study that application of troxerutin improves hippocampal LTP and reduces the amount of oxidative stress markers in the hippocampus of Aβ-treated rats (unpublished). Our study also revealed that i.c.v. injection of Aβ (1-42) had no effect on the swimming speed and motor function of experimental animals, and impairment in the water maze task in Aβ-treated rats is criticially due to cognitive deficits.

In conclusion, troxerutin demonstrated a clear protective effect on learning and memory impairment in the Aβ (1-42)-treated AD-like animal model. This effect may be partly mediated by abilities of the examined agent to exert antioxidant and anti-inflammatory effects, to improve hippocampal LTP, to modify the activity of cholinergic system, and to decrease the AChE activity and/or AGEs level in different sites of the brain, especially in the hippocampus. This study suggests that further studies are needed to elucidate the mechanism of action of troxerutin, a candidate for the prevention of AD and other neurodegenerative diseases.

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