EXPERIMENTAL STUDY

The effects and mechanisms of the action of galangin on spatial memory in rats

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ABSTRACT

BACKGROUND: Galangin, a flavonoid compound with acetylcholinesterase inhibitory activity, may improve cognitive functions by enhancing cholinergic transmission.

OBJECTIVES: We aimed to investigate the effects of galangin on spatial memory impairment in rats.

METHODS: The effects of galangin (50 and 100 mg/kg) and reference anti-dementia drug donepezil (1mg/kg) administrations were examined on memory impairment induced by the muscarinic cholinergic receptor antagonist scopolamine or the nicotinic cholinergic receptor antagonist mecamylamine in the Morris water maze (MWM) test. Hippocampal acetylcholine concentrations were also determined.

RESULTS: Galangin 50 and 100 mg/kg significantly decreased the mean distance to platform and increased the time spent in the escape platform quadrant in scopolamine-treated rats. Galangin 100 mg/kg significantly decreased the mean distance to platform and increased the time spent in the escape platform quadrant in mecamylamine-treated rats. The effects of galangin in the MWM were comparable with donepezil. Scopolamine and mecamylamine decreased acetylcholine concentrations, whereas galangin both alone and with mecamylamine or scopolamine administration increased acetylcholine concentrations.

CONCLUSION: Galangin improved memory impairment comparable to donepezil and nicotinic and muscarinic receptors may be involved in this effect. Galangin may be considered as a promising flavonoid in the prevention and treatment of memory impairment in Alzheimer's disease and other dementias *(Fig. 7, Ref. 37)*. Text in PDF *www.elis.sk*.

KEY WORDS: spatial memory, cognition, galangin, Morris water maze, rats.

Introduction

It is estimated that there are 24.3 million people with dementia worldwide and this number will be about 81.1 million by 2040. Alzheimer's disease is the most frequent cause of dementia, followed by vascular disease (1, 2). Dementia is characterized by a decline in cognitive functions, primarily in learning and memory, behavioral alterations, and decreased daily functioning (3). The late stages of the disease bring deteriorations in spatial functions and self-management. It was shown that the hippocampal region was associated with spatial learning and memory in the brain (4). This region has a unique role in memory functions by connecting different components of experiences about life (5).

The cholinergic system, with its complicated network in the central nervous system is essential for maintaining cognitive, emotional, and behavioral functions normally (6). In addition, this system is one of the most important neurotransmitter systems functioning in spatial learning and memory (7). The functions of cholinergic system are mediated by acetylcholine through muscarinic and nicotinic receptors (8).

Cognitive impairments are associated with many neurodegenerative diseases such as: Alzheimer's and Parkinson's disease (9). Enhancing cholinergic transmission in the brain produces improvements in cognitive functions, especially in learning and memory. Inhibition of acetylcholinesterase, which catalyzes the degradation of acetylcholine, is one of the strategies for enhancing cholinergic transmission (10). Currently, acetylcholinesterase inhibitors donepezil, galantamine, and rivastigmine are approved and commonly used for symptomatic treatment of cognitive dysfunctions in Alzheimer's disease and other dementias (11, 12). Researchers are focused on developing new agents with a cholinesterase-inhibiting effect. Galangin (3,5,7-trihydroxyflavone) is a flavonoid found in *Alpinia officinarum*. Flavonoids are phytochemicals beneficial for human health, which play significant roles in various biologic processes and have been center of interest in scientific researches (13, 14). The antiproliferative, anticancer, and anti-inflammatory features of galangin have been reported (15, 16). In addition, the acetylcholinesterase-inhibiting activity of galangin was reported with the highest inhibitory activity among studied 21 flavonoids (17).

In this study, we aimed to investigate the effects of acute galangin administration on spatial memory and mechanisms involved in rats in the Morris water maze (MWM) test. The MWM is a test used to investigate spatial learning and memory, it is superior to other tests that assess learning and memory (18, 19). For this purpose, galangin was administered both alone and with a nicotinic cholinergic receptor antagonist, mecamylamine or with a muscarinic cholinergic receptor antagonist, scopolamine. In addition, the effects of galangin on hippocampal acetylcholine concentrations were also studied. The effects of galangin in the MWM were compared to donepezil, which was used as a reference anti-dementia drug.

Material and methods

Experimental Design and Groups

The effects of acute galangin administration were investigated on mecamylamine or scopolamine-induced memory impairment using a spatial navigation task in the MWM in rats. Mecamylamine, a nicotinic cholinergic receptor antagonist or scopolamine, a muscarinic cholinergic receptor antagonist, were used to induce memory impairment and also to assess possible involving muscarinic or nicotinic cholinergic mechanisms in the effects of galangin. To compare the effects of galangin in MWM, donepezil was used as a reference drug, which has acetylcholinesterase inhibitor activity and is approved for Alzheimer's disease and other dementias (20). The groups were composed as below:

In naive rats (unimpaired memory):
  1. Control group (n = 8)
  2. Galangin 50 mg/kg group (n = 8): Galangin 50 mg/kg
  3. Galangin 100 mg/kg group (n = 8): Galangin 100 mg/kg

In mecamylamine-treated rats:
  1. Control group (n = 8): vehicle injected group
  2. Mecamylamine group (n = 8): mecamylamine 7.5 mg/kg
  3. Galangin 50 mg/kg group (n = 8): Galangin 50 mg/kg + mecamylamine 7.5 mg/kg
  4. Galangin 100 mg/kg group (n = 8): Galangin 100 mg/kg + mecamylamine 7.5 mg/kg
  5. Donepezil group (n = 8): Donepezil 1 mg/kg + mecamylamine 7.5 mg/kg

In scopolamine-treated rats:
  1. Control group (n = 8): vehicle injected group
  2. Scopolamine group (n = 8): scopolamine 1.5 mg/kg
  3. Galangin 50 mg/kg group (n = 8): Galangin 50 mg/kg + scopolamine 1.5 mg/kg
  4. Galangin 100 mg/kg group (n = 8): Galangin 100 mg/kg + scopolamine 1.5 mg/kg
  5. Donepezil group (n = 8): Donepezil 1 mg/kg + scopolamine 1.5 mg/kg

Acute galangin or donepezil administrations were performed in the retention test. Mecamylamine or scopolamine were administered 30 minutes after galangin or donepezil injections and then the rats were immediately released to the pool for the retention test.

Animals

Male Sprague Dawley rats (250–300 g) were used in the study. Animals were sheltered in standard conditions of light (12 hours light / dark cycle) and temperature (21 ± 1 °C). Food and water were available *ad libitum*. All the experiments were performed with the permission of the Local Ethics Committee of Eskisehir Osmangazi University for the care and use of laboratory animals (Date and number of the permission: 27-02-2013 / 317).

Drugs

Galangin (Alfa Aesar) were dissolved in distilled water: DMSO (5:1 ratio) and administered intraperitoneally. Donepezil hydrochloride monohydrate (Sigma-Aldrich) and mecamylamine hydrochloride (Santa Cruz Biotechnology) were dissolved in saline and injected subcutaneously. Scopolamine hydrobromide trihydrate (Acros Organics) were dissolved in saline and given intraperitoneally.

Morris Water Maze (MWM)

The MWM is the test used for evaluating spatial learning and memory (19). Test was performed according to literature (21). *Time spent in the escape platform quadrant (*s) and the *mean distance to platform (cm)* were recorded and analyzed using a video tracking system (Noldus Ethovision XT, Version 9, Wageningen, The Netherlands). The "mean distance to platform" means "the mean of the distance to the quadrant, in which the platform is positioned during a 60-s probe trial performance". If this value was smaller, it means that the rat swam closer to the escape platform during the probe test". The time spent in the escape platform quadrant and the mean distance to the platform calculations were used as measures for the development of spatial memory.

Determination of hippocampal acetylcholine concentrations

After the retention test, rats were sacrificed immediately and their hippocampal regions were dissected out for the determination of acetylcholine concentrations. Tissues were stored at –80 °C until the determination of acetylcholine concentrations. Acetylcholine concentrations were determined using an enzyme-linked immunosorbent assay, which was performed in accordance with the instructions of the commercial kit (EnzyChromTM Acetylcholine Assay Kit (EACL-100), BioAssay Systems, CA, USA).

Statistical analysis

The SPSS Version 21 program was used for a statistical analysis. Data were statistically analyzed using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test. In addition, the Tamhane test was used for multiple comparisons. $P < 0.05$ was accepted as statistically significant.

Results

The effects of galangin on unimpaired memory in naive rats in MWM

Galangin at both doses, 50 and 100 mg/kg, did not alter the *time spent in the escape platform quadrant* and the *mean distance to*
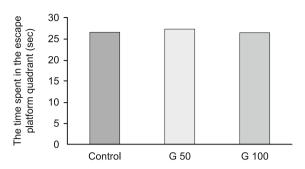
Fig. 1. The effects of galangin on the time spent in the escape platform quadrant in naïve rats. Results are given as the median (25–75 % percentiles). G 50: Galangin 50 mg/kg, G 100: Galangin 100 mg/kg.
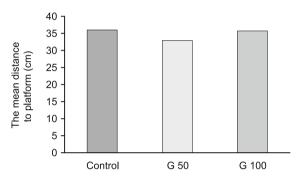

Fig. 2. The effects of galangin on the mean distance to platform in naïve rats. Results are given as the median (25–75 % percentiles). G 50: Galangin 50 mg/kg, G 100: Galangin 100 mg/kg.
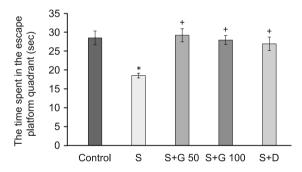

Fig. 3. The effects of galangin on the time spent in escape platform quadrant in rats with scopolamine-induced memory impairment. Results are given as the mean ± SEM. * p < 0.05, compared with the control; + p < 0.05, compared with the scopolamine group. S: Scopolamine, S+G 50: Scopolamine+Galangin 50 mg/kg, S+G 100: Scopolamine+Galangin 100 mg/kg, S+D: Scopolamine+Donepezil.

platform compared to the control, when it was administered alone without scopolamine or mecamylamine, which induce memory impairment in rats (p > 0.05) (Figs 1 and 2, respectively).

The effects of galangin on scopolamine-induced memory impairment in MWM

There was a significant decrease in the *time spent in the escape platform quadrant* and a significant increase in the *mean distance to platform* in scopolamine alone-injected rats compared

to the control group. Galangin at both doses of 50 and 100 mg/kg and donepezil 1 mg/kg significantly increased the *time spent in the escape platform quadrant* and decreased the *mean distance to platform* in scopolamine-injected rats compared to scopolamine alone-injected rats (p < 0.05) (Figs 3 and 4, respectively).

The effects of galangin on mecamylamine-induced memory impairment in MWM

There was a significant decrease in the *time spent in the escape platform quadrant* and a significant increase in the *mean distance to platform* in mecamylamine alone-injected rats compared to the control group. Galangin at the 100 mg/kg dose and donepezil 1 mg/kg significantly increased the *time spent in the escape platform quadrant* and decreased the *mean distance to platform* in mecamylamine-injected rats compared to mecamylamine alone-injected rats (p < 0.05) (Figs 5 and 6, respectively).

The effects of galangin on hippocampal acetylcholine concentrations in rats

Scopolamine or mecamylamine significantly decreased acetylcholine concentrations compared to the control (Fig. 7). Both
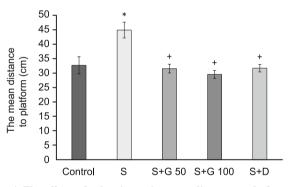

Fig. 4. The effects of galangin on the mean distance to platform in rats with scopolamine-induced memory impairment. Results are given as the mean ± SEM. * p < 0.05, compared to the control; + p < 0.05, compared to the scopolamine group. S: Scopolamine, S+G 50: Scopolamine+Galangin 50 mg/kg, S+G 100: Scopolamine+Galangin 100 mg/kg, S+D: Scopolamine+Donepezil.
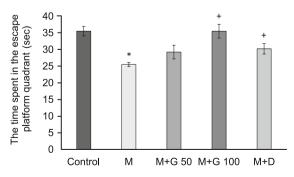

Fig. 5. The effects of galangin on the time spent in the escape platform quadrant in rats with mecamylamine-induced memory impairment. Results are given as the mean ± SEM. * p < 0.05, compared to control; + p < 0.05, compared to mecamylamine group. M: Mecamylamine, M+G 50: Mecamylamine+Galangin 50 mg/kg, M+G 100: Mecamylamine + Galangin 100 mg/kg, M+ D: Mecamylamine +Donepezil.
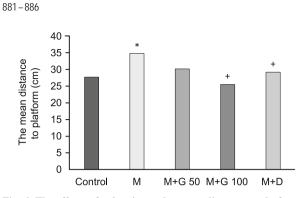
Fig. 6. The effects of galangin on the mean distance to platform in rats with mecamylamine-induced memory impairment. Results are given as the median (25–75 % percentiles). * p < 0.05, compared to the control; + p < 0.05, compared to the mecamylamine group. M: Mecamylamine, M+G 50: Mecamylamine+Galangin 50 mg/kg, M+G 100: Mecamylamine+Galangin 100 mg/kg, M+ D: Mecamylamine+Donepezil.
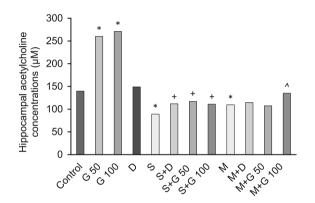

Fig. 7. The effects of galangin alone or in combination with scopolamine or mecamylamine on hippocampal acetylcholine concentrations. G 50: Galangin 50 mg/kg, G 100: Galangin 100 mg/kg, S: scopolamine, M: mecamylamine, S+G 50: Scopolamine+Galangin 50 mg/kg, S+G 100: Scopolamine+Galangin 100 mg/kg, M+G 50: Mecamylamine+Galangin 50 mg/kg, M+G 100: Mecamylamine+Galangin 100 mg/kg. * p < 0.05, compared to the control, + p < 0.05, compared to scopolamine ^ compared to the mecamylamine group.

galangin doses increased acetylcholine concentrations, when administered with scopolamine compared to the scopolamine alone group (Fig. 7). In addition, galangin 100 mg/kg dose increased acetylcholine concentrations, when administered with mecamylamine, compared to mecamylamine alone group (Fig. 7). Also, galangin at both doses of 50 and 100 mg/kg increased acetylcholine concentrations compared to the control in treatment-naïve rats that did not take scopolamine or mecamylamine injections (Fig. 7). In this aspect, we may suggest that galangin may be preventive for memory impairment.

Discussion

In this study, we investigated the effects of acute galangin administration on scopolamine or mecamylamine-induced memory impairment in the MWM in rats. We observed that galangin

prevented memory impairment induced by both scopolamine and mecamylamine. These effects were comparable to the reference drug donepezil, which is used in the treatment of dementia disorders. In addition, galangin increased hippocampal acetylcholine concentrations, when administered alone and also prevented the decrease in hippocampal acetylcholine concentrations induced by scopolamine or mecamylamine.

Alzheimer's disease is one of the most common causes of dementia (22). Although, there are acetylcholinesterase inhibitors that are used for the treatment of cognitive dysfunction in dementia, researchers are focused on developing more effective acetylcholinesterase inhibitors (23).

Galangin is a flavonoid compound that is commonly used in traditional medicine (24). Many bioactivities of galangin such as: antiinflammatory, anticancer, and antiviral effects have been reported (25). In addition, based on the studies reporting the potent acetylcholinesterase inhibitory effect of galangin, it was suggested that galangin might be a potential treatment option in Alzheimer's disease and other dementias (17, 23, 26, 27). Low brain acetylcholine concentrations, especially in the hippocampus, are associated with cognitive dysfunction and increasing acetylcholine concentrations in the brain via inhibition of acetylcholinesterase, which is accepted as a helpful way of treating the cognitive dysfunction seen in Alzheimer's disease and other dementias (28, 29). In this study, galangin prevented the memory impairment induced by scopolamine or mecamylamine. These results may suggest a potential role for galangin in the treatment of dementia. Moreover, a preventive role for galangin may be suggested in memory impairment considering its enhancing effect on hippocampal acetylcholine concentrations, when administered alone. In contrast to our results, galangin showed no beneficial effect on D-galactose-induced cognitive dysfunction in mice (30). However, this study differs from our study in terms of the research method and the animals. In our study, we induced memory impairment by administering the muscarinic acetylcholine receptor antagonist scopolamine or nicotinic acetylcholine receptor antagonist mecamylamine to rats. Scopolamine is reported to inhibit cholinergic functions in the central nervous system and increase the activity of acetylcholinesterase (31, 32).

Galangin prevented both scopolamine and mecamylamine-induced memory impairment. Thus, we may suggest that galangin exerted this effect by affecting muscarinic and nicotinic acetylcholine receptors. This dual effect of galangin on both acetylcholine receptors may be explained by the increased acetylcholine concentrations in the hippocampus. We observed that acute galangin administration increased hippocampal acetylcholine concentrations at both doses of 50 and 100 mg/kg, when administered alone without scopolamine or mecamylamine injections. This is in accordance with the reported acetylcholinesterase inhibitory effect of galangin (17). In addition, galangin increased hippocampal acetylcholine concentrations, when administered in combination with scopolamine or mecamylamine. Moreover, it was reported that galangin exerted a neuroprotective effect in an Alzheimer's disease model and researchers suggested a potential role for galangin in its treatment (33). Additionally, it was observed that galangin inhibited

experimentally induced neuroinflammation and considered it as a candidate agent in the treatment of neuroinflammatory diseases such as: Alzheimer's and Parkinson's disease (34). These studies present data supporting our results showing the beneficial effect of galangin on memory impairment.

Dementia diseases are one of the most common health and socioeconomic problems in the elderly population worldwide (35). Acetylcholinesterase inhibitors are first-line treatment options in Alzheimer's disease and other dementias (36, 37). In this study, we compared the effects of galangin with the acetylcholinesterase inhibitor donepezil, which is commonly prescribed in dementia and observed that galangin is comparable to donepezil.

Conclusion

Galangin may improve memory impairment. Both nicotinic and muscarinic receptors may be involved in this effect. We suggest that galangin may be a potential promising agent in the prevention and treatment of cognitive dysfunction.

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