Elderly Health Journal 2017; 3(1): 50-54.
Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Journal Website : http://ehj.ssu.ac.ir

Original Article

Effects of Different Coumarin- 3-Carboxamide Agents on Scopolamine Induced Learning and Memory Deficit in Mice

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A B S T R A C T

Article history
Received 19 Apr 2017
Accepted 2 June 2017

Introduction: It has been shown that three new synthetic coumarins-3-carboxamides including 3-fluorobenzilchloride, 4-fluorobenzilchloride and 2-hidroxy-3 metoxybenzaldehyde, have acetylcholinesterase inhibitory activity. This study was performed to estimate ameliorating effect of these new coumarin-3-carboxamides on memory impairments induced by scopolamine (1 mg/kg, induced prolongation) in mice.

Methods: 30 male mice were divided into five groups, 6 mice in each group. Three experiment groups received coumarins-3- carboxamides (10 mg/kg body weight) 30 min before scopalamin injection and two other groups considered as normal (saline-treated) groups and finally one negative control (scopalamin only) group. The experiment groups were treated with coumarins of 3-fluorobenzilchloride, 4-fluorobenzilchloride and 2-hidroxy-3 metoxybenzaldehyde. The passive avoidance test was performed in an automatic conventional shuttle box set-up. The stepped down latency and number of errors was recorded.

Results: With reference to saline-treated group, scopolamine-treated mice demonstrated impairment of learning and memory as a reduction of latency and an increased numbers of errors in step-down test)$p < 0.01$). Treated mice receiving these coumarins at the dose of 10 mg/kg showed an increase in the number of avoidances on the memory tests compared to the scopolamine group ($p < 0.01$).

Conclusion: The study has demonstrated some therapeutic effects of coumarin-3-carboxamides on learning and memory deficit induced by scopolamine. Further investigation is needed to explore whether coumarin-3-carboxamides could be beneficial for memory impairment in Alzheimer's disease in which cholinergic deficit is one of the hallmarks.

Keywords: Coumarins, Scopolamine, Passive Avoidance, 3-fluorobenzilchloride, 4-fluorobenzilchloride, 2-hidroxy-3 metoxybenzaldehyde

Citation: Ghanei Nasab S, Hadizadeh F, Marjani A. Effects of Different Coumarin- 3-Carboxamide Agents on Scopolamine Induced Learning and Memory Deficit in Mice. Elderly Health Journal. 2017; 3(1): 50-54.

Introduction

The increase in life expectancy during the 20th century had concomitantly increased the number of people suffering of age related diseases. Dementia, a clinical syndrome characterized by the development of multiple cognitive deficits that are severe enough to interfere with daily functioning, has become a major public health issue (1, 2). It is imposing a tremendous economic impact on both affected individuals and the entire society (3). The most common form of dementia among older people is Alzheimer's disease (AD) (4). The pathophysiology of AD is complex and involves several different biochemical pathways. The key symptoms of AD are primarily caused by cholinergic dysfunction. It is known that acetylcholine (ACh) is an important neurotransmitter related to memory and learning (5). Based on a cholinergic

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[DOR: 20.1001.1.24236179.2017.3.1.8.5]

hypothesis, attempts were conducted to reverse cognitive deficits by increasing brain cholinergic activity via acetylcholinesterase (AChE) inhibitors (6). Coumarins are an important class of natural compounds and are used as additives in both foods and cosmetics (7). Coumarins has been reported to have antibacterial (8), anti-oxidant (9), anti-inflammatory and anticoagulant (10), and anti-AD activities (11). Coumarins moiety is an important aromatic ring with a broad spectrum of biological activity (12). Among the various classes of compounds studied for design of new AChE inhibitors, coumarins scaffold has received great attention (13). Recently, we have reported different series of coumarin-based AChE inhibitors (14). Previous studies have described the usefulness of indole amine framework in the design of new AChE inhibitors or multi-target agents for AD therapy. Luo et al. synthesized a series of indole amine-based benzylpyridinium bromides that showed anti-AD with cholinesterase inhibitory, antioxidant, and neuroprotective activities (15). In this context and following to our recent works (14), it seems very interesting to develop some coumarins carboxamide. We reported here the anti-dementia effect of new coumarins agents including 3-fluorobenzilchloride, 4-fluorobenzilchloride and 2-hidroxy-3 metoxybenzaldehyde against scopolamine induced learning and memory deficit in mice. Previous study showed that these synthesized agents have cholinesterase inhibitory, antioxidant, and neuroprotective activities (15). To our knowledge, nothing is known about the effect of these new coumarins on memory using male animal models. So, we evaluated the effect of single dose of these agents on male mice with memory impairment induced by scopolamine using the passive avoidance test.

Methods

Animals

30 Male mice, weighing 25-30 g, were obtained from Laboratory Animal Center, Shahid Sadoughi University of medical sciences, Yazd, Iran. Prior to testing, animals were housed, 6 mice per cage for one week. They were allowed free access to water and food ad libitum, and maintained in a constant temperature (22 ± 1°C) environment under a 12-h light/dark cycle.

Treatment

To study the effects of coumarin-3-carboxamides on impairment of learning and memory induced by scopolamine. Five groups (n = 6) of animals were used: 1 group received scopolamine only, as negative control group and another group received normal saline as normal group. The remaining 3 groups were treated with the coumarin-3-carboxamides (3-fluorobenzilchloride, 4-fluorobenzilchloride and 2-hidroxy-3 metoxybenzaldehyde, 10 mg/kg body weight) 30 minutes before scopolamine (1mg/kg body weight) administration. Behavioral tests were carried out at 30 minutes after the injection of scopolamine (16).

Step-down test

A step-down passive avoidance was examined using apparatus consisted of plexiglass chamber. The inside dimensions of the activity cage were, length 35 cm; width 23 cm; and height 20 cm. The cage floor was made of evenly spaced stainless steel bars (3 mm diameter) that were spaced 11 mm apart, and a plastic platform (5 cm diameter, 4 cm height) set on the grid in one corner. Electric stimulation was given through the grid connected with a scrambled shock generator (1 Hz, 1 ms, 36 V dc). Mouse was allowed to get adapted to environment in the cage for 3 minutes without electric shock. Then it was placed on the platform, electric shocks were delivered to the grid when the mouse stepped down from the platform. The electric shocks were still delivered for 5 minutes. After 24 hours of training, mouse was placed on the platform for retention test. The electric shocks were still delivered for 5 minutes. Step-down latency, the time that elapsed until the mouse stepped down from the platform, and the number of errors was recorded. If the mouse did not step down from the platform within 300 seconds, the retention test was terminated and the maximal step-down latency of 300 seconds was recorded. An error was counted whenever the mouse stepped down from the platform and the number of errors made in 5 minutes was record.

Ethical considerations

The study protocol was approved by institutional review board in Arak branch, Islamic Azad University, Arak, Iran. All the necessary ethical issues in use of animals in research considered throughout the study.

Data analysis

All data were expressed as the mean value for the group ± standard error of the mean. Statistical analyses were performed by one-way ANOVA and Tukey's post-hoc test for planned comparisons between control versus different treatment groups. A value of $p < 0.05$ was considered as statistically significant.

Results

Effects of coumarin-3-carboxamides on step-down passive Avoidance test

We assessed whether coumarin-3-carboxamides could improve memory function in passive avoidance learning. As shown in Figure 1 and 2, no differences in either initial step-down latency or numbers of error were noted among different treatment groups in acquisition test.

[DOR: 20.1001.1.24236179.2017.3.1.8.5]

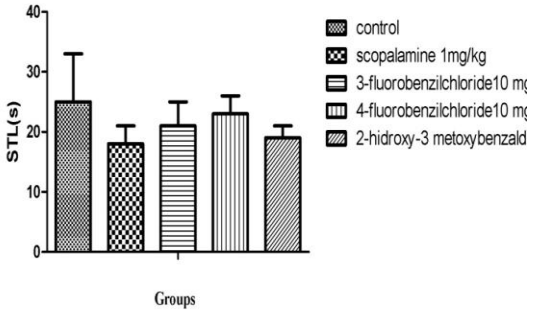

Figure 1. The effect of coumarin-3-carboxamides on scopolamine-induced memory impairment on step-down latency (s) in initial the step-down avoidance test. Mice received an injection of vehicle (control group) or scopolamine (1.00 mg/kg, in SCOP coumarin-3-carboxamides treated groups) 30 min before the training session.
SCOP: scopolamine
STL: step-through latency

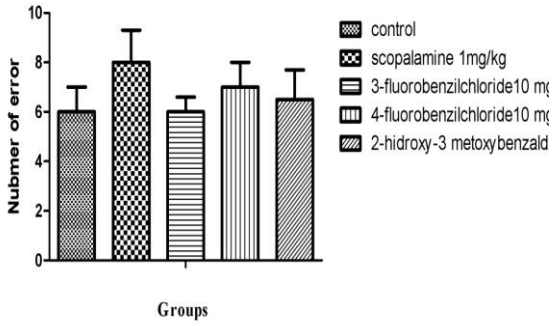

Figure 2. The effect of coumarin-3-carboxamides on scopolamine-induced memory impairment on the number of errors in initial the step-down avoidance test. Mice received an injection of vehicle (control group) or scopolamine (1.00 mg/kg, in SCOP coumarin-3-carboxamides treated groups) 30 min before the training session. Values are presented as medians and interquartile ranges.
SCOP: scopolamine

However, on the second day, scopolamine (1 mg/kg body weight) significantly shortened the step-down latency and increased numbers of error when compared to saline treated group ($p < 0.05$). All coumarin-3-carboxamides significantly effect on step-down latencies or number of errors when compared to scopolamine-treated group (Figure 3 and 4).

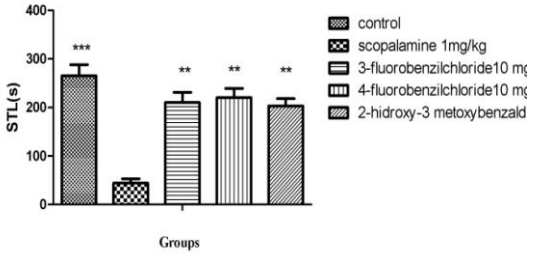

Figure 3. The effect of coumarin-3-carboxamides on scopolamine-induced memory impairment on step-down latency (s) in the step-down avoidance test 24 hour after training session.

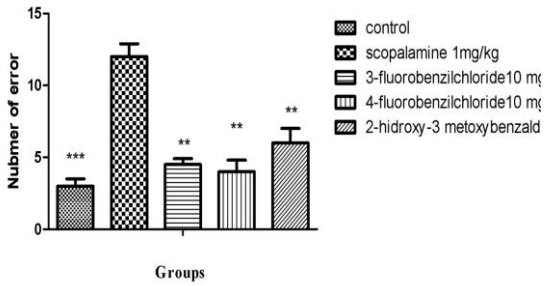

Figure 4. The effect of coumarin-3-carboxamides on scopolamine-induced memory impairment on the number of errors in the step-down avoidance test. 24 hour after training session.

Discussion

In this study, memory deficit was induced by the administration of scopolamine which interferes memory and cognitive function in experimental animals by blocking muscarinic receptors (16). Scopolamine has been extensively used to screen for drugs with potential therapeutic value for dementia (17). Longer escape latency in a shorter step-down latency than those exhibited by mice in control group were noted in scopolamine-treated mice (18). As a result, there is a growing interest in demonstrating relationships between consumption of natural products and risk reduction and/or prevention of various disease and health conditions. AD is the major form of dementia and is a deadly neurodegenerative disease that is progressive in nature and develops through a multifactorial process. Although its etiology remains unknown, the neuropathological profile of AD is associated with memory loss and is consistent with the presence of numerous plaques and cholinergic deficiency due to the degeneration or atrophy of cholinergic neurons in the basal forebrain (19). With respect to the regulation of cognitive functions, the central cholinergic system is considered to be the most important neurotransmitter (20). Indeed, cholinesterases such as AChE and Butyrlcholinesterase are considered key enzymes that play significant roles in cholinergic transmission by hydrolyzing the neurotransmitter ACh (21). Several of the cholinesterase inhibitors currently in use, namely donepezil, tacrine, rivastigmine, and galantamine, have adverse side-effects such as gastrointestinal disturbances, nausea, vomiting, and diarrhea and also have bioavailability issues (22). For these reasons, there is growing scientific interest in identifying natural sources of AChE, inhibitors with safer profiles. To evaluate the potential of the new synthetic coumarins as anti-AD agents, we investigated their ability to act as anti-dementia, which was induced by scopolamine in mice. Our results showed that these agents could have preventive effects against scopolamine impairment memory. Various active compounds and extracts obtained from medicinal plants have been assessed for their efficacy against AD (23). Use of a single molecule for disease treatment and research is often preferred in order to better understand the mechanism of action. Our in

[DOR: 20.1001.1.24236179.2017.3.1.8.5]

vivo findings corresponded with the acetylcholine esterase inhibitory activity of these coumarins. In our previous study the best result was obtained with 4-fluorobenzilchloride against AChE, displaying IC50 value of 0.16 mM. The structure–activity relationship study demonstrated that the introduction of benzyloxy moiety on the 7-position of coumarins scaffold can improve the anti-AChE activity. The in vivo study also showed that this compound has best effect against memory deficiency induced by scopolamine in mice however this difference is not significant (14).

Conclusion

In summary, our synthesis coumarins have a significant ability to prevent dementia induced by scopolamine. The results of the present study suggest that these coumarins may be good candidates for development as therapeutic agents for the treatment and prevention of AD by targeting b-amyloid formation. Further cellular based studies are needed to help clarify the detailed mechanism of action of these compounds in the brain membrane and other organs.

Study limitations

The authors acknowledge all people who have assisted in the study procedure in School of medicine, Shahid Sadoughi University of medical sciences and, Arak branch, Islamic Azad University.

Conflict of interest

We certify that no actual or potential conflict of interest related to this article exists..

Acknowledgment

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