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Research Article
Effect of Warfarin Treatment on Memory Function in Mice

Ahmad H. Alhowail, Syed I. Rabbani, Ibrahim F. Alharbi, Hindi S. Alharbi and Raed S. Alharbi

Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, 51452 Al-Qassim, Kingdom of Saudi Arabia

Abstract

Background and Objective: Warfarin is widely used in the treatment of stroke and other thromboembolic diseases. The effect of warfarin on memory function is not clear; however, warfarin is a vitamin K inhibitor. This study aimed to test the effect of warfarin on memory function in mice. **Materials and Methods:** Twenty mice (18-30 g) were divided into control and warfarin-treated groups; each group contained 10 animals. The warfarin-treated group received warfarin dissolved in drinking water at 0.05 mg mL^{-1}. Animals were treated for 9 days and monitored for mortality. On the day 7 of treatment, the animals were subjected to behavioral tests using the Y-maze, novel object recognition and elevated plus maze tests. **Results:** In the Y-maze test, warfarin-treated animals showed a slight increase in the number of entries into the novel arm and the time spent there. The time spent exploring the novel object and the transfer latency was increased in the novel object recognition test and the elevated plus maze test, respectively. **Conclusion:** This study suggested that warfarin treatment affected the memory function of mice. The mechanism of the cognitive loss could involve depletion of vitamin K in the body.

Key words: Warfarin, memory function, Y-maze, elevated plus maze, vitamin K, cognitive diseases, thromboembolic disease

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Corresponding Author: Ahmad H. Alhowail, Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, 51452 Al-Qassim, Kingdom of Saudi Arabia

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INTRODUCTION

Cardiovascular complications are the leading cause of mortality worldwide. Currently available therapies play a significant role in improving the life-expectancy of patients. However, morbidity due to the disease sometimes goes unnoticed, resulting in disorders in the later part of life[1]. Stroke is a cardiovascular disease that occurs due to thromboembolism and treatment is generally started immediately. The most commonly used drugs for the prevention of stroke and other thrombus-related diseases are anticoagulants[2].

Warfarin is an essential anticoagulant drug that is used for the prevention of blood clot formation as well as treatment of thrombosis following stroke[3]. Warfarin is a vitamin K antagonist that acts to inhibit the formation of vitamin K by inhibiting the enzyme vitamin K epoxide reductase[4]. Warfarin has a narrow therapeutic range, which means it must be given with caution. Although there are established dosing guidelines, warfarin therapy is reported to be associated with a number of adverse effects and drug-drug interactions[5]. The relationship between warfarin and cognitive function is not well identified in the literature. However, long-term treatment with warfarin has been reported to cause vitamin K deficiency[6].

The exact role of vitamin K in cognitive function and learning and memory processes is still not fully clear. However, study by Brangier *et al.*[7] revealed that chronic use of vitamin K antagonists results in cognitive decline in older people. Another study revealed that vitamin K antagonists increase the risk of cognitive impairment[8]. Several studies have indicated a role of vitamin K in impairment of cognitive function[9]. However, the role of warfarin on the memory function is not well documented in the literature. Considering the importance of warfarin in the treatment of thrombotic diseases, this study was designed to evaluate the effect of sub-chronic warfarin treatment on memory function using multiple behavioral tests such as Y-maze, novel object recognition and elevated plus maze in mice.

MATERIALS AND METHODS

The study was conducted in the research lab of Pharmacology and Toxicology department, College of Pharmacy, Qassim University. The study was conducted for the duration of 2 weeks in the month of September, 2019.

Animals: Twenty mice (10-12 weeks old, about 18-30 g) were individually housed and maintained in a 12- h:12-h light/dark cycle (lights on 6:00 am). Water and food were provided. The mice were divided into treatment and control groups (10 mice per group). The animals were observed daily for any changes and mortality. All behavioral tests were performed during the light time of the cycle. The experiment was conducted after obtaining permission from the Research Unit, College of Pharmacy, Qassim University (Approval ID 2019-CP-8).

Drug administration: Warfarin sodium was obtained from Crescent Pharma as 5 mg tablets. The mice were treated with warfarin orally by dissolving it in the drinking water at 0.05 mg mL^{-1}. The concentration of the drug in water was determined from the daily water intake of the animals and by modifying the reported dose of warfarin in mice so that it could be administered for 9 days with minimal toxicity[10]. Warfarin was given daily for 9 days and control animals received only saline. After that, the animals were subjected to behavioral tests.

Assessment of spatial memory using Y-maze: The Y-maze test assesses the ability of animals to distinguish places that they have already explored and their propensity to explore new places. The Y-maze was made using wood and had dimensions of 40×8×12 cm in each arm. Each of the 3 arms was at 120°C to the other arms and was painted brown for easy visualization during tests. The Y-maze apparatus was placed on the floor. A light was placed above each arm to ensure equal light distribution. A camera and software were used to record all test sessions. The Y-maze test evaluates the working and spatial memory of the mice by providing cues at the end of each arm. Parameters such as the number of entries into the novel arms and the time spent in the novel arms were recorded per the procedure described in Valentim *et al.*[11].

Novel object recognition test: The NOR test is a hippocampal-dependent memory test that measures spatial memory function. The apparatus was a cube made of wood with an open top. The size of the box was 40×40×40 cm. The objects used in this test were 2 white teacups and the novel test object was a rectangular metal box of similar size to the teacups. In this test, the mice were introduced to the teacups for 5 min for familiarization. Then, the animals were returned to their cages. Three hours later, the animals were examined for 5 min. During the examination session, one of the teacups

was replaced with the rectangular metal box. The time spent exploring the novel object was recorded using a camera set above the apparatus and analyzed[12].

Elevated plus maze test: The EPM test is a behavioral test used to measure memory function[13]. The maze was composed of 2 open and 2 closed arms. The length of the arms was 30 cm, with width 5 cm. The height of the walls in the closed arms was 15 cm. The central area between the 4 arms was 5 cm^2. The maze was placed on a stand that elevated it above the floor by about 30 cm.

During the training session, each mouse was placed individually in an open arm. The mice were allowed to explore the apparatus for 5 min. Three hours later, each mouse was placed in same place as in the training session but for examination. The latency time is defined as the time that the mouse took to enter a closed arm. The animal was considered to have entered the arm when all its legs entered the arm. A video camera was placed above the maze to record the examination session. The latency time was scored and compared between warfarin-treated and control mice[13].

Statistical analysis: Behavioral data from this study were analyzed using unpaired t-tests. All experiments were repeated for n = 5 animals in each group and a p-value<0.05 was considered statistically significant.

RESULTS

Mortality: There was a higher incidence of death among the mice that received warfarin (Fig. 1); 50% of the warfarin-treated mice died within 9 days, even though the dose used was within the normal range.

Effect of warfarin on Y-maze test: This study examined the effect of warfarin administration on Y-maze tasks. According to the result it revealed that saline and warfarin groups did not induce significant change in total number of entering the novel arm (Fig. 2). In addition, there were no differences in the total spent time of the novel arm observed among the 2 groups (Saline and warfarin) (Fig. 3). Therefore, result indicated that there was no effect of warfarin on Y-maze tasks.

Effect of warfarin in the NOR test: The result of NOR test indicated that there were no significant changes induced by warfarin in the novel object recognition test. Therefore, the result of NOR illustrated that warfarin not altered the memory function in treated mice (Fig. 4).

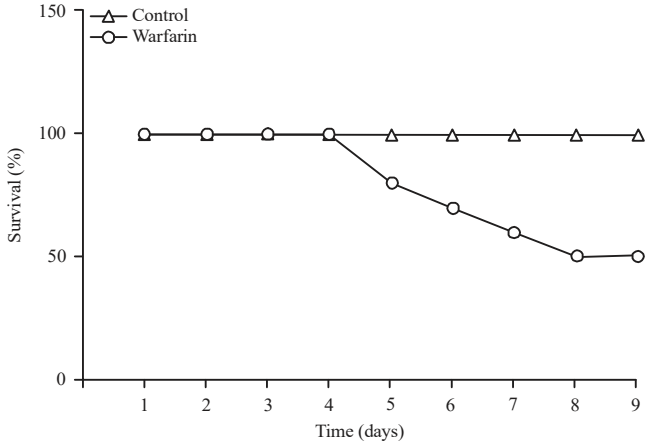

Fig. 1: Warfarin reduced the survival rate of treated mice
Warfarin treatment was administered orally by dissolving it in drinking water at 0.05 mg mL^{-1}

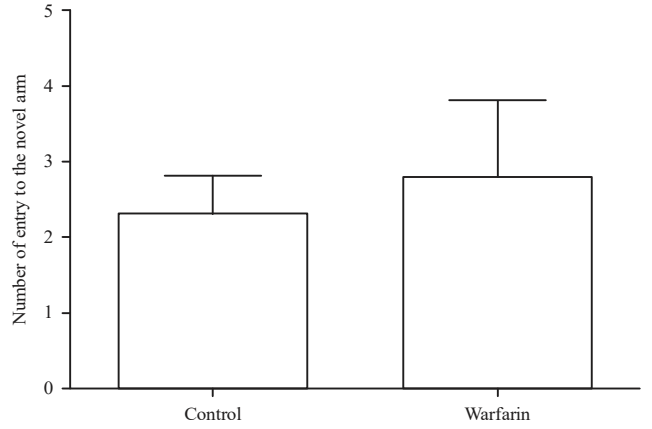

Fig. 2: Y-maze test performance
Warfarin-treated mice showed no significant cognitive deficit compared with control mice

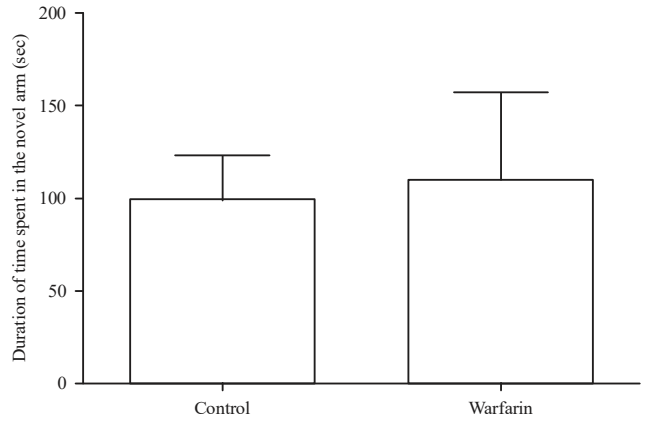

Fig. 3: Effect of warfarin on the time spent in the novel arm in the Y-maze

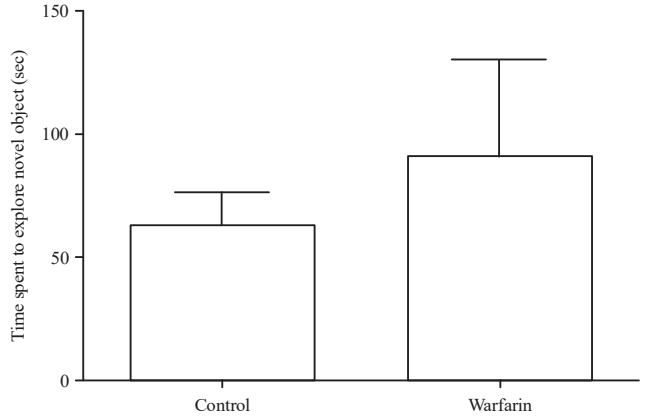

Fig. 4: Novel object recognition test results
No significant difference was found between the warfarin-treated and control groups

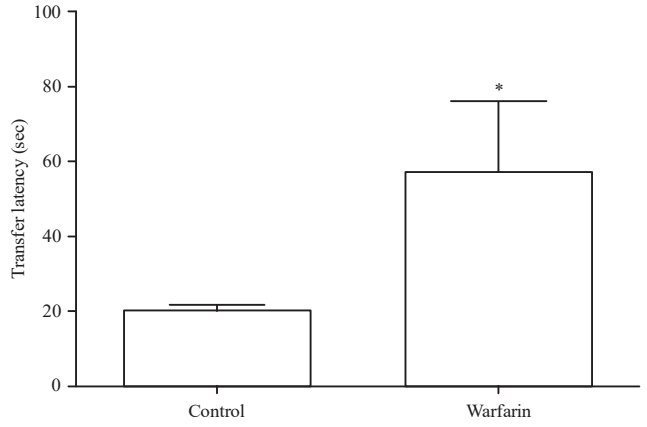

Fig. 5: Elevated plus maze test performance
*Warfarin-treated mice had higher transfer latency times than the non-treated (control) mice. This test indicates a memory impairment in the warfarin mice compared with the control mice

Effect of warfarin on the EPM test: In the warfarin group (dissolved on drinking water at concentration of 0.05 mg mL^{-1}), the latency time (LT) increased on the 9th day, indicating that the memory was impaired compared to the saline group. Therefore, warfarin induced impairment in the memory at EMP tests (Fig. 5).

DISCUSSION

In the present study, impairment of cognitive function due to the administration of warfarin was tested using models including the Y-maze and EPM. These test systems are commonly employed to study the influence of treatment and diseased states on cognitive functions, including memory[14]. In the Y-maze behavior test, the willingness of the rodents to explore a new environment is evaluated. The normal behavior of rodents is to investigate a new environment, rather than returning to previously discovered areas[15]. This study observations indicated that the warfarin-treated animals showed an increase in the number of entries into the novel arm (26%) and the duration of time spent in the novel arm was enhanced in comparison with control animals (Fig. 2 and 3). When animals are tested, these parameters usually decrease, suggesting that the animals remember previous experiences in the novel areas[15]. However, this study indicated that warfarin treatment had a negative effect on this cognitive function.

The NOR test assesses exploratory behavior of animals[16]. The test evaluates cognition such as memory in rodent models of central nervous system disorders; the choice of the animals to explore the novel object is reported to indicate the use of learning and recognition memory[12]. In the EPM experiment, rodents show the natural behavior of escaping into the closed arms, as they dislike open and high spaces. The time the animals take to move from an open arm into a closed arm is recorded as the transfer latency[13]. The data in this study indicated that warfarin-treated animals spent 38.4% more time exploring the novel object in the NOR test and their transfer latency was enhanced by 186% (Fig. 5). Exploratory behavior of the animals involves different parts of the brain, including the hippocampus, basal forebrain, septum and prefrontal cortex[16]. Increase in transfer latency and in the time spent exploring the novel objects are indicators of impaired memory[14].

Coronary artery diseases have been reported to be associated with cognitive decline in patients. Patients who have suffered stroke show impairment in memory, orientation, verbal skills, visuospatial ability and abstract reasoning. Multiple mechanisms, such as cerebral microbleeds, cerebral hypoperfusion and intracranial and extracranial hemorrhage, have been suggested for the defects[17]. In addition, elevation of the levels of inflammatory markers such as C-reactive protein, tumor necrosis factor-alpha and interleukin-6 have been linked to the cognitive decline[18].

Oral anticoagulants play a crucial role in the management of stroke and can decrease the stroke rate by nearly 50% if the therapy is planned optimally[19]. Studies have reported that warfarin did not affect memory function in patients[3]. However, long-term exposure to warfarin has been reported to cause deficiency of vitamin K in the body[4]. Vitamin K is a group of structurally similar, fat-soluble vitamins found in food and is essential for the synthesis of blood clotting factors. It is found in two natural vitamers, vitamin K1 (phylloquinone) and vitamin K2 (menaquinone); vitamin K1 is acquired from plants, while bacteria present in the gut convert[20] vitamin K1 to vitamin K2.

Studies have indicated that vitamin K is involved in sphingolipid metabolism[9]. Sphingolipids are a class of lipids that involved in the proliferation, differentiation and survival of neurons. Alteration in the production of sphingolipids can cause neuroinflammation and neurodegeneration[9]. Further, it is reported that vitamin K has antiapoptotic and anti-inflammatory effects that are mediated through the activation of Growth Arrest Specific Gene-6 and Protein S[21]. From these findings combined with present data, it can be speculated that subchronic warfarin treatment in current study depleted vitamin K, leading to neuroinflammation and neurodegeneration that might have impaired memory function in the mice. In support of this, studies have reported that warfarin treatment produced symptoms of dementia in elderly patients[7]. More research in this direction is suggested to determine the precise mechanisms of warfarin in memory loss and cognitive impairment.

CONCLUSION

Subchronic treatment with warfarin resulted in an increase in parameters indicating memory impairment in mice. Warfarin being a vitamin K antagonist might have interfered with the normal functioning of vitamin K in the central nervous system, leading to cognitive defects. More studies are suggested to determine the effect of warfarin on brain functions as this drug is widely used for the treatment of thromboembolic disorders.

SIGNIFICANCE STATEMENT

The findings from this study discovered that 2 weeks treatment of warfarin affected the memory parameters in mice such that the treatment increased the number of entries to novel arm, duration of time spent in novel arm, time spent to explore the novel object and transfer latency. The study revealed that the longer duration of warfarin therapy in animals such as mice could modulate the cognitive functions. These effects of drugs that occurs during normal course of treatment goes unnoticed and are not well documented but has the tendency to adversely affect the prognosis of the therapy. Considering the therapeutic applications of anticoagulants such as warfarin, the findings of this study could provide a platform for future research to explore the role of warfarin on the memory function and cognitive impairments in the various animal models and human subjects.

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