

Effect of metformin on the spatial memory in aged rats

Zobeideh Ashroostaghi¹, Farzaneh Ganji^{1,2}, Hamid Sepehri²

¹Department of Biology, Faculty of Science, Golestan University, Gorgan, Golestan, Iran.

²Neuroscience Research Center, Golestan University of Medical Sciences, Gorgan, Golestan, Iran.

Correspondence to: Farzaneh Ganji, E-mail: ganji.f@gmail.com

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ABSTRACT

Background: Aging process is often accompanied with some degree of decline in all the abilities, including learning and memory. One of the attracting research fields has been devoted to finding antiaging drugs. Metformin has shown some memory-enhancing features in aged humans and laboratory animals. **Aims and Objective:** To evaluate the effects of 50, 75, and 100 mg/kg of metformin on the spatial memory performance of aged rats in the Morris water maze. **Materials and Methods:** Thirty-two male 24-month-old rats were divided randomly into four groups ($n = 8$) including control group and 50-, 75-, and 100-mg/kg metformin groups. After 36 days of treatment, the learning process was assessed by the reference memory task in the Morris water maze. All the rats received water maze training (four trials/day for 5 days) to assess the hippocampal-dependent spatial learning and, then, received a 60-s probe trial test of spatial memory retention 24 h after the twentieth trial. **Result:** Over 5 days of training, metformin (50, 75, and 100 mg/kg/day) treatment significantly reduced the latency and path length to find the escape platform ($P < 0.01$). In probe trials (without platform), on the last day of training, the metformin-treated groups spent significantly longer time in the platform quadrant when compared with the control group. Among the treated groups, 100 mg/kg dosage of metformin induced the best rehearsals memory ($P < 0.05$). **Conclusion:** These results showed that, in the old rats, 36-day orally administered metformin showed a positive influence on the spatial memory performance in the Morris water maze.

KEY WORDS: Aging; Spatial Memory; Metformin; Morris Water Maze


INTRODUCTION

A decline in the abilities, including cognition, has been considered as the inevitable consequence of the aging process. Aging is characterized by cognitive decline, which actually presents as one of the formidable features of aging.^[1,2] Age-related deficits in different kinds of memory including spatial learning and memory are widely reported.^[1,3,4] A behavioral model often used to study the relationship between cognitive decline in rodents is spatial

learning.^[5] Of the various behavioral functions, Morris water maze (MWM) enables the analysis of spatial learning in rodents, which depends on distal cues to find a way from the beginning point around the perimeter of an open swimming pool to identify the escape platform that is under water.^[5,6] Several studies have confirmed its effectiveness in determining the spatial navigation that relies on the hippocampus.^[7]

The hippocampus is one of the brain areas involved in spatial learning and memory.^[8] It has been suggested that the age-related decline in spatial-learning abilities is owing to the changes in the function and morphology of the hippocampal formation.^[9]

In the recent years, many research works have been devoted to find drugs to postpone the beginning of cognitive decline in elderly persons. For example, to boost the spatial memory, a leading antidiabetic drug (i.e., metformin) has been examined and suggested.^[10,11] A long-term treatment with metformin has been estimated to improve the health and lifespan in mice.^[12]

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This study was undertaken to investigate the effects of 50, 75, and 100 mg/kg of metformin on the spatial learning of aged rats using MWM task.

MATERIALS AND METHODS

Twenty-four-month-old rats weighing 400–450 g were obtained from the Aging Farm. They were housed two per cage in a temperature- (23 ± 1°C) and light (12-h light/dark schedule; lights on at 8:00 a.m.)-controlled environment and fed laboratory food and water ad libitum. All the protocols for the experiments on the animals were approved by the Research and Ethics Committee of Golestan University of Medical Sciences, Golestan, Iran. The animals were randomly divided into four groups of eight animals in each, including control group and 50-, 75-, and 100-mg/kg metformin groups, respectively. Metformin was purchased from Sigma-Aldrich, dissolved in saline, and administered via oral route for 36 days. Beginning on day 37, the spatial memory of rats in all the four groups was evaluated by MWM task.

Morris Water Maze Task

The MWM used in our study was a black circular pool (160 cm diameter, 60 cm high) filled with water (30 cm depth) at 24 ± 2°C. The pool was divided into four quadrants arbitrarily designed northeast (NE), northwest (NW), southeast (SE), and southwest (SW) areas. A submerged Plexiglas platform (10 cm × 10 cm) was hidden 1 cm below the water surface and placed in a constant location in the center of NW quadrant. The animals received 5 days of training with the hidden platform; each day included four training sessions with a 60-s intersession interval. Each trial was started by placing a rat with its face toward the wall of the pool at one of the three start points. The start location was varied on each training trial and changed each day. The trial was terminated when the animal entered the platform. If the rat did not find the platform within 60 s, it was placed on the platform by the experimenter for 15 s. During acquisition of the spatial navigation task, all the groups were given one session of four trials each day (days 1–5; trials 1–20). The spatial memory was evaluated in the probe trial. On the sixth day (trial 21), the platform was removed, and the animals were allowed to swim for 60 s. The path of the animals in the maze was monitored using a computerized video tracking system (Maze router, Urmia Instruments Inc). Parameters measured were the time taken to reach the platform (latency), swimming speed, and swim path length (SPL) in the training trials. Moreover, during the probe trials, the running time percentage within the quadrant of the water bath where the hidden platform had been placed in the training trials was calculated for each experimental animal.

Statistical Analysis

Data were analyzed using SPSS software, version 11.5, and plotted as mean ± SD. Comparison among the groups was

made using analysis of variance (ANOVA) with a post hoc Tukey test or repeated-measures ANOVA. The results were considered significantly different when $P < 0.05$ and highly significantly different when $P < 0.01$.

RESULT

The escape latency and the distance traveled by the rats to finding the hidden platform in the water maze task are presented in Figures 1 and 2, respectively. The ability of all the experimental animals to find the platform progressively improved over the 5 days of acquisition ($P < 0.05$).

However, repeated-measures ANOVA of these data revealed that the performance on this task differed between the control group and each of the three metformin-treated groups in the first four trial days ($P < 0.01$). Inspection of the data in these days showed that animals in the metformin-treated groups learned the task at a high rate, traveled a shorter distance, and spent less time to find the escape platform than the control group ($P < 0.01$); but, on the final trial day, the escape latency and path length were similar in all the experimental groups. Furthermore, in days 3 and 4, the 50-mg/kg dose of metformin showed better results when compared with the 75-mg/kg dose of metformin. But, the most effective dose of metformin for decreasing the escape latency and SPL in days 2, 3, and 4 of the trials was 100 mg/kg ($P < 0.01$). The analysis of swimming speed by using two-way ANOVA also showed no significant difference as the training days progressed among the groups and no interaction between the days and the groups [Figure 1C].

Probe Trial

Retention of the spatial training was assessed 24 h after the last training session with a 60-s free-swim probe trial using a new starting position. The parameters measured on the probe trial were the time spent in the quadrant containing the platform during training (target quadrant). Post hoc analysis showed that the metformin-treated groups showed significantly different experimental parameters from the control ones ($P < 0.05$, $P < 0.01$). The metformin-treated animals spent more time in the training quadrant when compared with the control animals in the 60-s probe trial [Figure 2]. In addition, among the treated groups, the 100-mg/kg/day group spent more time in the training quadrant ($P < 0.01$). Figure 3 shows representative plots (top view) of individual swim paths of one rat for each treatment group. It is obvious that, in the 100-mg/kg metformin group, the animal has spent most of its time in the target quadrant.

DISCUSSION

The results of this study suggest a spatial memory-enhancing role for metformin. Animals were treated for 36 days with three

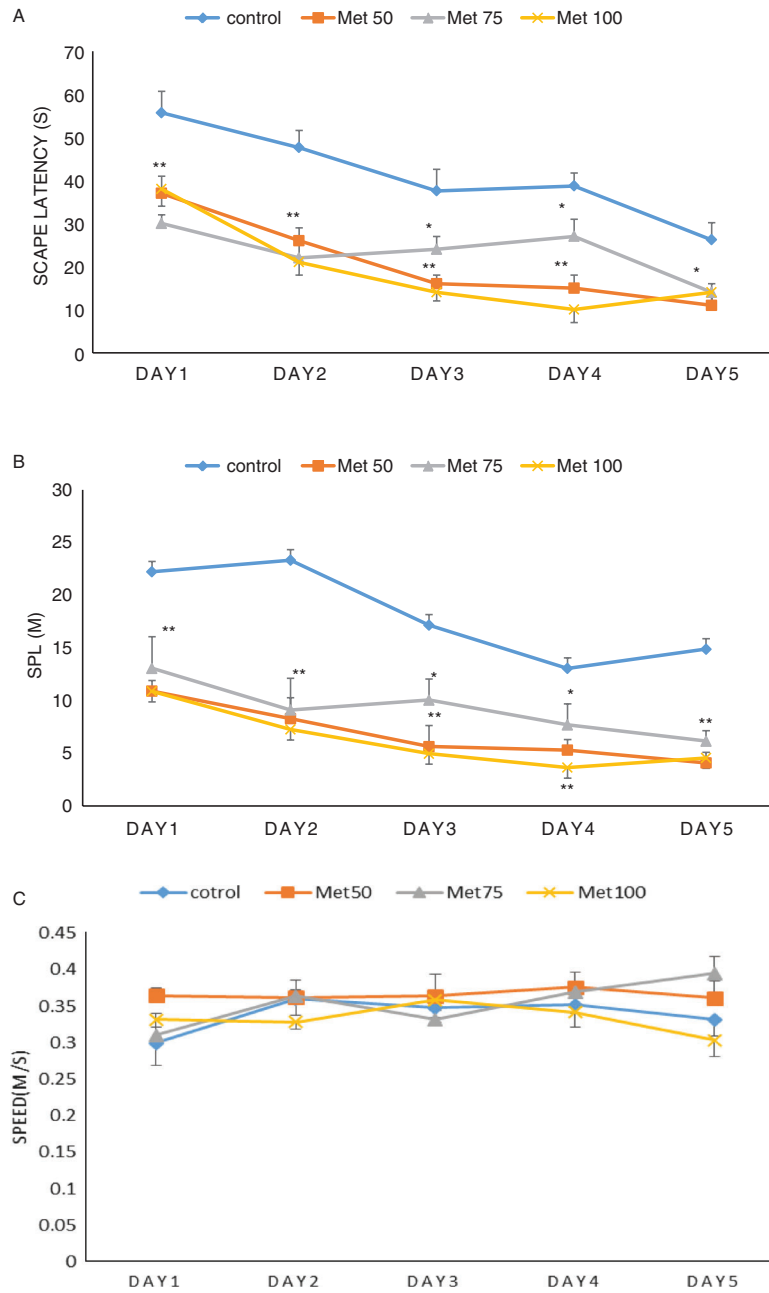


Figure 1. Effect of 36 days of treatment with metformin on the performance of spatial memory acquisition phase in Morris water maze. Average escape latency (A), distance traveled (B) within each day (made up of four trials), and swimming speed within each day (made up of four trials) (C) are shown. Asterisks indicate a significant difference from the control group (* $P < 0.05$, ** $P < 0.01$).

different doses of metformin, and their performance in a reference memory task, MWM, was compared with controls. The rats treated with 50-, 75-, and 100-mg/kg/day doses of metformin performed better than the control group in the water maze task. The latency and path length to finding the escape platform significantly reduced in the metformin-treated animals. Surprisingly, on the first day of testing, the

metformin-treated rats found the platform significantly faster than the control animals, and they were consistently better at the task than the control group until the fifth day of the trial.

Some recent research reports suggest that adult hippocampal neurogenesis is involved in hippocampus-mediated learning and memory. The two adult neurogenic regions, namely, the subventricular zone and the dentate gyrus show, in particular, a

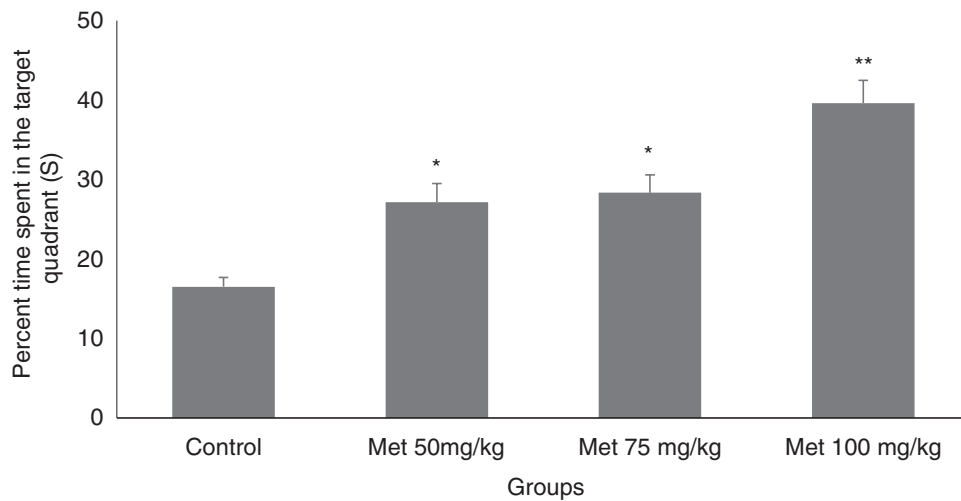


Figure 2: Mean percentage (\pm SEM) of the time spent in target quadrant during probe trials. Asterisks indicate a significant difference from control (* $P < 0.05$; ** $P < 0.01$).

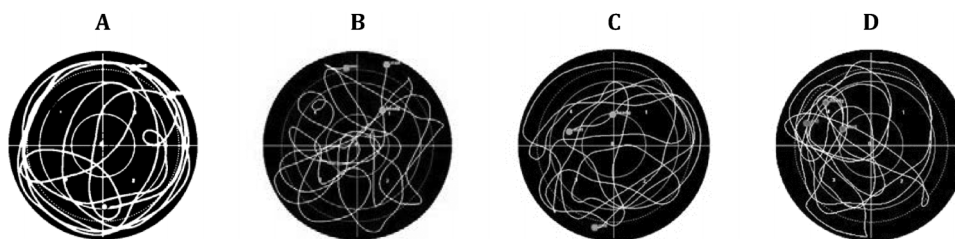


Figure 3: Tracings of the typical swim patterns in the probe trials at the control (A) and 50- (B), 75- (C), and 100-mg/kg (D) metformin groups tested. The escape platform was on northwest quadrant.

reduction in neurogenesis that is related to aging.^[13] Metformin has increased the number of neurons generated in the dentate gyrus of rats,^[14] and Wang *et al.*^[10] have shown that metformin improves the ability to update new spatial memories, a task related to hippocampal neurogenesis. It seems reasonable that, in our experiments, metformin even at the dose as low as 50 mg/kg/day for 36 days could stimulate hippocampal neurogenesis and prevent the spatial memory loss to some degree. On the other hand, it is a well-established fact that oxidative stress mechanisms have a prominent role in the aging process and make the neurons vulnerable to degeneration and development of neurodegenerative disorders.^[15] Some researchers have proposed an antioxidant role for metformin.^[16,17] Perhaps, metformin with its antioxidant effects could be beneficial in preventing oxidative damage to the hippocampal neurons and improve the animals' performance in the MWM task. The third possible mechanism of memory-enhancing feature of metformin may act via its interaction with brain neurotransmitter systems, especially cholinergic system. Bhutada *et al.*^[18] showed that metformin inhibits acetylcholine esterase and, so, it can potentiate acetylcholine effect on the hippocampus cholinergic synapses.

CONCLUSION

A 36-day treatment with 50, 75, and 100 mg/kg/day doses of metformin significantly improved the MWM task performance of aged rats, and the 100-mg/kg dose showed better results when compared with the two other doses. Exploring the exact mechanism of metformin memory-enhancing effect needs widespread research work.

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