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Glucosamine alleviates scopolamine induced spatial learning and memory deficits in rats

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Abstract

Introduction and objective: Beneficial effects of glucosamine in spatial learning and memory impairment induced by scopolamine has been evaluated in rats by using Morris water maze. **Methods:** Male Wistar rats were randomly divided into control, scopolamine and scopolamine plus glucosamine groups. All injections were given in 5 consecutive days and 30 min after each injection, the rats were tested in the Morris water maze test. Escape latency and path length to reach the hidden platform were subjected to analysis of variance [ANOVA]. **Results:** The rats treated with scopolamine showed increased escape latency and path length to reach the hidden platform compared to control group ($P < 0.001$). Both escape latency and traveled path length to reach the hidden platform in glucosamine treated animals (1 and 2 g/kg) were significantly lower ($P < 0.05$ to $P < 0.001$) than in the scopolamine group. **Conclusion:** The results of this study showed that the glucosamine can inhibit scopolamine-induced impairments of spatial learning and memory in rats. Glucosamine might offer a promise in either the prevention or the treatment of neurodegenerative diseases such as Alzheimer's disease.

Keywords: Scopolamine; D-Glucosamine; Morris water maze; Spatial learning and memory; Male rats

1. Introduction

Oxidative stress and reactive oxygen species lead to complex biochemical alterations and seem to be involved in memory and cognitive impairments present in aging and neurodegenerative disorders, including Alzheimer's disease (AD) [1].

Alzheimer is a common neurodegenerative disease that is characterized by memory loss and cognitive impairment. The pathological features of AD are senile plaques, development of neurofibrillary tangles, inflammation, neurotransmitter defect and oxidative stress [2,3]. Cholinergic dysfunction is one of the most prominent features of AD and the reduction in cholinergic activity is associated with the severity of the memory loss [4]. Increasing evidence imply that age-related oxidative stress has an important role in degenerative changes in basal forebrain cholinergic systems. Therefore, it seems that retarding the oxidative stress could be a suitable strategy to impede the progression of this disease [5].

D-Glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GLcNAc) are naturally occurring amino sugars and essential carbohydrate components of biologically important glycoproteins, glycolipids, and glycosaminoglycans. GlcN has been reported to have therapeutic potential in the treatments of various diseases including osteoarthritis, inflammatory bowel disease and gastritis [6–8]. GlcN has also been found to possess excellent antioxidant activities as manifested by strong chelating effect on ferrous ions and protection of macromolecules such as protein, lipid, and deoxyribose from

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oxidative damage induced by hydroxyl radicals [9,10]. In addition, recently the neuroprotective effect and also potential immunoregulatory capacity of glucosamine has also been suggested [10–12]. However, other biological activities of GlcN and its effects on spatial learning and memory have not been documented so far.

In the present study, the possible beneficial effect of glucosamine on spatial learning and memory impairment induced by scopolamine in rats was evaluated using Morris water maze.

2. Materials and methods

2.1. Animals and experimental protocol

Twenty eight male Wistar rats, (8 weeks old and weighing 200 ± 20 g) were maintained in constant temperature (22 ± 2 °C) with a relative humidity and 12 h light/dark cycle [light on from 06:00 to 18:00]. The ethical guidelines for the investigation of experimental animals were followed in all tests. All efforts were made to minimize the number of animals used and their suffering. They were randomly divided into four groups (n = 6–8 in each) and treated according to the experimental protocol.

Control rats received saline instead of both glucosamine and scopolamine before Morris water maze sessions. Group 2 (scopolamine; Sco) received saline instead of glucosamine and were injected by scopolamine [Sigma Chemical Co., USA] 30 min before Morris water maze sessions. In Group 3 (scopolamine + glucosamine 1 g; Sco + GlcN 1 g) and 4 (scopolamine + glucosamine 2 g; Sco + GlcN 2 g) were treated with 1 or 2 g/kg of glucosamine respectively, 30 min before each injection of scopolamine and finally, all animals were examined in Morris water maze. The volume of injection was 8 ml/kg.

2.2. Morris water maze apparatus and procedures

Morris water maze test was selected to evaluate spatial learning and memory [13,14]. A circular black pool (136 cm diameter, 60 cm high, 30 cm deep) was filled with water (23–24 °C). A circular platform (10 cm diameter, 28 cm high) was placed within the pool and was submerged approximately 2 cm below the surface of the water in the center of the southwest quadrant.

Outside the maze, fixed visual cues were present at various locations around the room (i.e., a computer, hardware and posters). Before each experiment, each rat was handled daily for 3 days and thus was habituated to the water maze for 30 s without a platform. The animals performed four trials on each of the five consecutive days, and each trial began with the rat being placed in the pool and released facing the side wall at one of four positions [the boundaries of the four quadrants, labeled North (N), East (E), South (S) and West (W)]. Release positions were randomly predetermined for each trial by computer. The rats were allowed to swim until they found and remained on the platform for 15 s. If 60 s had passed and the animals had not found the platform, they was guided to the platform and allowed to stay on the platform for 15 s. The rat was then removed from the pool, dried and placed in its holding bin for 5 min. The time latency to reach the platform and the length of the swimming path were recorded by a video tracking system [15–18].

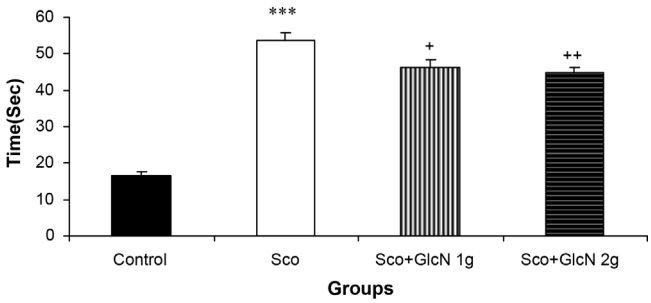

Fig. 1. Comparison of time latencies between groups. Rats in control group (n = 8) received saline injection. Animals in scopolamine (Sco) group (n = 6) were injected by scopolamine 30 min before Morris water maze test. In scopolamine + glucosamine 1 g (Sco + GlcN 1 g) and scopolamine + glucosamine 2 g (Sco + GlcN 2 g) groups (n = 6 in each group) the animals were treated by 1 and 2 g/kg of glucosamine respectively, before scopolamine and then were tested in Morris water maze. The injections were given for 5 consecutive days. Data are presented as means ± SEM. $^{***}P < 0.001$ compared to control group, $^{+}P < 0.05$ and $^{++}P < 0.01$ compared to scopolamine group.

2.3. Statistical analyses

The results were expressed as means ± SEM. The data of different groups over the five days of Morris water maze tests were compared using ANOVA test with Tukey's post hoc analysis between groups. Statistically significant differences were defined at $P < 0.05$.

3. Results

The Morris water maze task was carried out for five consecutive days to investigate the spatial learning and cognitive function of the rats treated with glucosamine. The escape latencies are shown in Fig. 1. Time latency to reach the hidden platform in the scopolamine group was significantly higher than in the control group ($P < 0.001$; Fig. 1). The animals of both the Sco + GlcN 1 g and Sco + GlcN 2 g groups had significantly shorter time latencies compared with the scopolamine group ($P < 0.05$ and $P < 0.01$; Fig. 1). Also, there was no significant difference between Sco + GlcN 1 g and Sco + GlcN 2 g groups in time latency.

The traveled path length to reach the hidden platform in the scopolamine group was significantly longer than in the control group ($P < 0.001$; Fig. 2). The animals of both the Sco + GlcN 1 g and Sco + GlcN 2 g groups had significantly shorter traveled path length compared to the scopolamine