

Research Article

MINOCYCLINE IMPROVES MEMORY IN MORRIS WATER MAZE TASK IN INTRACEREBROVENTRICULAR STREPTOZOTOCIN INFUSED RATSVivek Kumar Sharma^{1*} and Ashok Kumar Goyal²¹Govt. College of Pharmacy, Rohru, Dist. Shimla-171207, Himachal Pradesh, India²Onkar College of Pharmacy, Sunam, Dist. Sangrur, Punjab, India*Corresponding author's E-mail: viveksharma_pharma@yahoo.co.in

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ABSTRACT

In the present study, the effect of minocycline was studied against intracerebroventricular streptozotocin induced spatial memory deficit. Male wistar rats were injected with intracerebroventricular streptozotocin bilaterally. The rats were treated chronically with Minocycline (10, 20 and 40 mg/kg, ip) for 17 days starting from day 1 of streptozotocin injection in separate groups. The spatial memory was evaluated using the Morris water maze task. The intracerebroventricular streptozotocin infused rats showed significant memory deficits and minocycline showed significant dose dependent improvement in the task. The study demonstrated the effectiveness of minocycline in preventing spatial memory deficit induced by streptozotocin and its potential in age related neurodegenerative disorders where memory impairment is involved such as Alzheimer's disease.

Keywords: Minocycline, spatial memory, ICV streptozotocin rats, Morris water maze.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with a mean duration of around 8.5 years between onset of clinical symptoms and death¹. AD is the most common cause of dementia in the elderly population. The most widely used treatment for Alzheimer's disease at present are acetylcholinesterase inhibitors, which aim to prolong normal cognitive function through increased synaptic activity, without providing neuroprotection. This treatment is only symptomatic and provides modest outcomes for patients².

The intracerebroventricular streptozotocin (ICV STZ) model of cognitive impairment has been likened to sporadic dementia of Alzheimer's disease in rats. It is characterized by cognitive impairment, impaired glucose metabolism³⁻⁵, oxidative stress⁶ and a decrease in cholinergic markers in the brain. Alzheimer's disease patients show a deficit in spatial memory⁷⁻⁹. The Morris water maze is one of the choice apparatus to investigate the spatial memory deficits in rodent models of AD¹⁰.

Minocycline (MIN) is a second-generation tetracycline and has been found to exert biological effects independent of their antimicrobial properties^{11, 12}. These include anti-inflammatory activities such as inhibition of iNOS¹³, up regulation of interleukin 10¹⁴ etc. Minocycline has been commonly used to treat pneumonia and acne, as well as infections of the skin, genital, and urinary systems¹⁵. Minocycline effectively crosses the blood-brain barrier due to its small (495 d) and lipophilic nature¹⁶ and has been shown to exert neuroprotective effects distinct from its bacteriostatic activity in animal models of cerebral ischemia¹⁷, Parkinson's and Huntington's disease¹⁸. Based on several encouraging evidences of Minocycline in neurodegenerative disorders [We have

shown the protective effect of Minocycline against ICV STZ induced oxidative stress in rats¹¹, the present study was designed to investigate the effect of Minocycline in i.c.v. streptozotocin induced cognitive dysfunction in rats.

MATERIALS AND METHODS

Streptozotocin was purchased from Sigma–Aldrich, USA. Minocycline Hydrochloride (Ranbaxy, India) was used and the drug solutions were prepared by suspending them in one or two drops of Tween 80 in normal saline and administered intraperitoneally.

Animals

The experiments were carried out in adult (7-8 months old) male wistar rats (230-250 g) obtained from animal house of Onkar College of Pharmacy, Sajuma, Punjab, (India). All animals were housed in rodent cages in the animal room where the temperature was maintained approximately at 24-25°C and relative humidity of 60-65% with 12 hours dark light cycle (lights on 06.00 - 18.00 h). The food in the form of dry pellets and water was made available ad libitum. All behavioural experiments were carried out between 10 AM and 4 PM. All experiments were performed as per the norms of ethical committee and the studies were approved and clearance obtained by the Institutional review board.

Experimental groups

After adapting to the new environment for at least 7 days, animals were divided into seven groups and each group comprised of 10 animals.

Group 1: Sham Operated (SH) (Sham-operated rats wherein the surgery was performed minus drilling of holes and placement of the cannula).



Group2: (SH+ aCSF) artificial cerebrospinal fluid (aCSF) was infused i.c.v. in a volume of 10 μ l in each ventricle on day 1 and 3.

Group 3: STZ Control (STZ+ normal saline, as vehicle for MIN) (Rats were infused with i.c.v. streptozotocin (3mg/kg) dissolved in aCSF in a volume of 10 μ l in each ventricle on day 1 and 3 and the animals were treated with normal saline containing as a vehicle of minocycline for 21 days.

Group 4: MIN10 (STZ+ MIN, 10mg/kg, ip) Rats infused with i.c.v. streptozotocin on day 1 and 3 and immediately after first streptozotocin infusion, treated with 10 mg/kg i.p Minocycline(MIN) for 21 days.

Group 5: MIN20 (STZ+MIN, 20mg/kg, ip) Rats infused with i.c.v. streptozotocin were treated with minocycline at doses of 20 mg/kg, i.p. respectively for 21 days following 1st streptozotocin infusion.

Group 6: MIN40 (STZ+MIN, 40mg/kg, ip) Rats infused with i.c.v. streptozotocin were treated with minocycline at doses of 40 mg/kg, i.p. respectively for 21 days following 1st streptozotocin infusion.

Group 7: Per se group of normal animals were treated with 40mg/kg i. p. of minocycline.

Intracerebroventricular (i.c.v.) infusion of streptozotocin

Male wistar rats weighing 230-250g were anaesthetized with ketamine (100mg/kg, ip) and xylazine (5mg/kg, ip).The head was placed in position in the stereotaxic apparatus and a midline saggital incision was made in the scalp. Two holes were drilled through the skull for placement of infusion cannulae into the lateral cerebral ventricles using following coordinates: 0.8mm posterior to bregma; 1.5mm lateral to saggital suture; 3.6 mm ventral from the surface of the brain. Streptozotocin was dissolved in artificial cerebrospinal fluid (aCSF):[147mM NaCl;2.9mM KCl;1.6mM MgCl₂;1.7mM CaCl₂ and 2.2mM dextrose (pH7.4)] and slowly infused (1 μ l/min) using Hamilton micro syringe in a volume of 10 μ l into each cerebral ventricle (bilateral i.c.v.) on day 1 and 3 [20,21]. After ICV injection followed by daily application of NeosporinR. The body weights were continuously monitored.

BEHAVIORAL ASSESSMENT

For the behavioural tests each group comprised of 10 rats and only one animal was tested at a time.

MORRIS WATER MAZE (MWM)

Spatial learning and memory of animals was tested in a Morris water maze¹⁹. It consisted of a circular water tank (180 cm diameter, 60 cm height) filled with water (25 \pm 1°C) to a 177 depth of 40 cm. A non-toxic water dispersible emulsion was used to render the water opaque. Four equally spaced locations around the edge of the pool (North, South, East, and West) were used as start

points, which divided the pool into 4 quadrants. An escape platform (10 cm in diameter) was placed in the pool 2 cm below the surface of water. The escape platform was placed in the middle of one of the randomly selected quadrants of the pool and kept in the same position throughout the entire experiment (north-west for this study). Before the training started, the rats were allowed to swim freely into the pool for 120 s without platform.

Each animal was subjected to four consecutive trials on each day with an interval of 5 min. During which they were allowed to remain on the platform for 20 sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition. Rats were subjected to acquisition trial for four consecutive days (days17, 18, 19 and 20), each trial having a ceiling time of 120s and a trial interval of approximately 30s. After climbing onto the hidden platform, the animals remained there for 30s before commencement of the next trial. The time taken to locate the hidden platform (latency in seconds) was measured. Twenty four hours after the acquisition phase, a probe test (day 21) was conducted by removing the platform. Rats were allowed to swim freely in the pool for 120 s and the time spent in target quadrant, which had previously contained the hidden platform, was recorded. The time spent in the target quadrant indicated the degree of memory consolidation which had taken place after learning^{20,21}.

STATISTICAL ANALYSIS

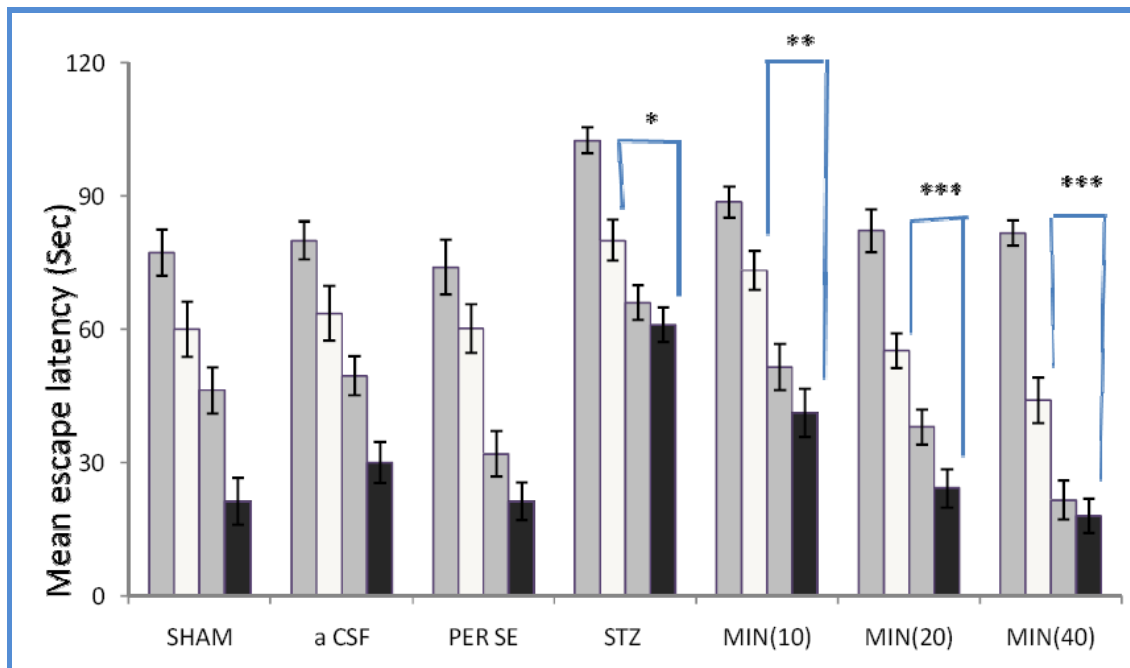
The results are expressed as means \pm S.D. Thebehavioural and biochemical values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P<0.05 was considered statistically significant.

RESULTS

Effect on Escape Latency Time (ELT) and Time Spent in Target Quadrant (Using Morris Water Maze) Minocycline (10 mg/kg, 20 mg/kg, 40 mg/kg, ip) showed dose dependent reduction on ELT in animals during acquisition trial conducted on day 1 to day 4. Minocycline (40 mg/kg, ip) significantly (p<0.05) prevented STZ induced increased ELT (Figure 1) when compared with respective STZ group. Minocycline (40 mg/kg, ip) also improved significantly (p<0.05) the time spent in target quadrant (Q4) in animals, in search of missing platform during retrieval trial conducted on day 5 (Figure 2) when compared with respective control group.



Figure 1: Effect of Minocycline on escape latency to find the hidden platform in morris water maze test in i.c.v. streptozotocin rats.

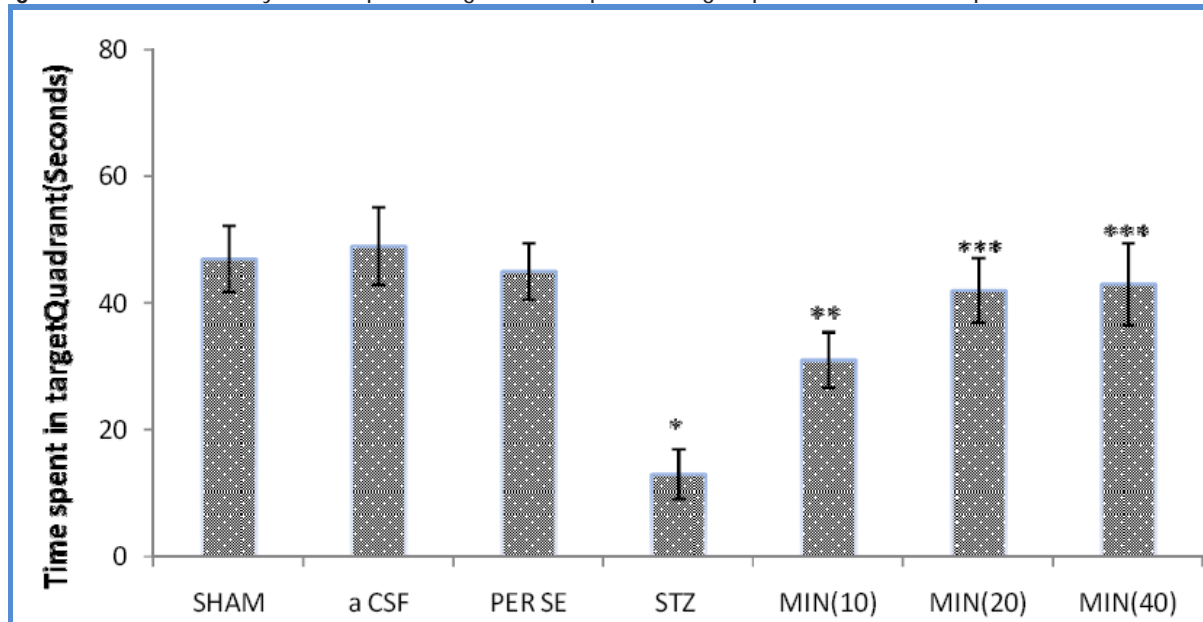


Mean escape latency to locate the hidden platform was recorded on days 17, 18, 19 and 20 to a maximum of 120s. Values are expressed as mean±S.D. (n=10). The mean escape latency (days 18–20) to find the hidden platform was significantly prolonged in i.c.v. streptozotocin group compared with sham group (* $P < 0.05$ vs sham group).

Minocycline significantly reversed streptozotocin induced learning deficit compared with streptozotocin group [$**P < 0.05$ vs i.c.v. streptozotocin group, $***P < 0.05$ vs i.c.v. streptozotocin and MIN 10 (Minocycline at 10 mg/kg) groups on days 18, 19 and 20].

Note — Each bar represents mean escape latency of four trials per day i.e. on days 17, 18, 19 and 20 respectively.

Figure 2: Effect of minocycline on percentage of time spent in target quadrant in i.c.v. streptozotocin infused rats.



Values are expressed as mean±S.D. (n=10). The percentage of time spent in target quadrant was significantly lesser in i.c.v. streptozotocin infused group compared with sham group (* $P < 0.05$ vs sham group). Minocycline treatment in i.c.v. streptozotocin infused rats significantly attenuated streptozotocin induced memory deficits compared with i.c.v. streptozotocin group [$**P < 0.05$ vs i.c.v. streptozotocin group, $***P < 0.05$ vs i.c.v. streptozotocin and MIN10 (Minocycline at 10 mg/kg) groups on day 21].

aCSF=artificial cerebrospinal fluid; Min10, Min20 and Min40 = Minocycline at 10, 20 and 40 mg/kg dose respectively, Per se = administered minocycline at a dose of 40 mg/kg in normal animals.

DISCUSSION

Since its first application in 1981, the Morris water maze has become one of the most frequently used tool for analyzing spatial learning and memory in rodents²². The task is based on the premise that animals have evolved an optimal strategy to explore their environment and escape from the water with a minimum amount of effort i.e., swimming the shortest distance possible. The time it takes a rat to find a hidden platform in a water pool after previous exposure to the set up, using only available external cues, is determined as a measure of spatial memory²³.

The hippocampus and the cerebral cortex are the key structures of memory formation. Because the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function²². The cognitive decline in AD is accompanied by neuronal atrophy and loss, mainly in the cortex hippocampus and amygdale²⁴ and Morris water escape task can test of the hippocampus in learning and memory and is widely were used to assess learning and memory particularly in neurodegenerative disorders like Alzheimer's disease²¹.

Although neurobehavioral deficits in animals are less directly translatable to humans than other pathological changes because behaviors tend to be highly species specific and spatial learning is a good example of behavior that rats and other rodents are particularly good at but is not generally as well developed or used as an end-point to assess cognitive function in humans. Therefore, direct extrapolation of results of a Morris water maze task is probably ill advised. Nevertheless, the underlying functional mechanisms of the brain and their involvement in behaviours are shared across most mammalian species. Therefore, changes in rat behavior are likely to model effects in humans where the same functions are shared, although the precise behaviours may be different. Thus Minocycline can be thought to be a future pipeline target where scientist will have to work to bring a sure cure for AD.

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