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Dual Effects of Aspirin on Spatial Learning and Memory of Male Rats Following Induction of Permanent Cerebral Ischemia

Narges Ashraf Ganjooei[1], Vahid Sheibani[1], Mandana Jafari[2], Zahra Hajializadeh[3], Ali Shamsara[4], Ali Dehghani[5], Mohammad Reza Afarinesh[1*]

[1]Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[2]Herbal and Traditional Medicines Research Center, Kerman University of Medical Sciences, Kerman, Iran
[3]Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[4]Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[5]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

*Corresponding Author: Mohammad Reza Afarinesh, Emails: reza.afarinesh@gmail.com and r.afarinesh@kmu.ac.ir

Abstract

Background: Ischemic stroke can lead to cognitive impairment, and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to slow down the progression of Alzheimer›s disease (AD). This study focused on rodent models to investigate the impact of ischemic stroke and the potential benefits of aspirin in reducing cognitive impairment.

Methods: The Morris water maze (MWM) was used to evaluate memory and learning in seven groups (N = 63) of Wistar rats. Brain ischemia was induced in rat models through temporary blocking of both common carotid arteries and permanent blocking of the middle cerebral artery (MCA). Aspirin 20, 40, and 80 mg/kg IP was administered 30 minutes and 2 hours after stroke induction in both ischemic and non-ischemic rats. Injections were continued for seven consecutive days in these groups, and learning and memory were evaluated after the last injection.

Results: Data analysis of the MWM test showed a significant increase in escape latency and swim path length to find the platform in the ischemic groups compared to control rats ($P<0.005$). Despite improvement in all experimental groups after intervention ($P<0.001$), the scores for spatial learning were significantly decreased by aspirin in no-ischemia + ASA groups compared to the control group ($P<0.05$). In the ischemia + ASA groups, aspirin at the dose of 20 mg/kg but not at the high dose (80 mg/kg) improved spatial learning compared to the control group.

Conclusion: Repeated treatments with aspirin may impair spatial learning and memory in normal rats, however, aspirin at a low dose of 20 mg/kg may improve learning impairment after ischemic stroke.

Keywords: Aspirin, Cerebral ischemia, Learning, Memory, Rats, Stroke

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Introduction

Brain injury resulting from stroke remains a major public health problem (1). Based on statistical information, the number of strokes diagnosed worldwide has increased annually over the past two decades. Stroke continues to be one of the most frequent causes of death and a major cause of permanent disability in many countries (2-4). Stroke is associated with a high incidence of sensory, motor, and cognitive deficits in humans (5). One common impairment after an ischemic stroke is memory impairment, which can be induced in animal models for conducting studies (6).

The middle cerebral artery (MCA) is the most commonly affected in strokes (7). Possible treatments and therapies for damage caused by stroke in humans can be studied by examining the behavioral and anatomical consequences of ischemia in rat stroke models prepared by cerebral arterial occlusion (8). Based on epidemiological investigation, it has been shown that nonsteroidal anti-inflammatory drugs (NSAIDs) have potential preventive or ameliorative effects on Alzheimer's disease (AD). The relative risk for developing AD is markedly lower in subjects under 85 years who also use NSAIDs for 6 months or more in the preceding year, suggesting the potential ameliorative role of NSAIDs in cognitive and neurological impairments (9). One of the most widely used NSAIDs is the o-acetyl derivative of salicylate, *i.e.*, acetylsalicylic acid or aspirin

(10).

Aspirin has a broad range of pharmacological actions, from pain and fever control to prevention and treatment of stroke (11). Aspirin may reduce the size of infarcts after ischemic stroke, which has been generally attributed to its antiplatelet action by inhibiting cyclooxygenase (COX)-dependent pathways (12). NSAIDs block prostaglandin formation, which is the basis for their anti-inflammatory and analgesic effects (13). In a process that relies on COX, prostaglandins are produced as metabolic byproducts enzymatically derived from the fatty acid arachidonic acid (13). The controversial findings regarding aspirin's impact on cognitive function suggest that its effects may not be limited solely to pain relief, prompting a closer examination of its influence on specific brain regions associated with learning and memory.

The hippocampus is a crucial brain region involved in learning, memory formation, and spatial navigation. Hippocampal function is very important in Morris water maze (MWM) performance (14). Therefore, MWM is a suitable tool for empirically assessing the effects of NSAID administration on cognitive function in ischemic rats. The previous study (15) showed that aspirin at a dose of 30 mg effectively decreases the degeneration of CA1 of the hippocampus following both transient and permanent middle cerebral artery occlusion (MCAO).

Some reports explain the inverse association between cognitive decline and NSAID use (16-18). In addition, some studies have found that while repetitive administration of aspirin after the onset of cerebral ischemia is not beneficial, administration of doses between 15 and 80 mg/kg of aspirin shortly before MCAO has neuroprotective effects (19). Previous studies have shown that injection of a single dose of aspirin at 30 mg/kg just 30 min after the onset of transient MCAO could improve spatial learning and memory (20,21).

Therefore, the present study aimed to test the possible neuroprotective effects of both immediate and repeated administration of aspirin after MCAO. Using a permanent MCAO rat model, the present study aimed to clarify if post-ischemic therapy with aspirin could improve spatial learning and memory recovery.

Methods

Animals

Adult male Wistar rats weighing 250 ± 50 g (age: 2-3 months) were used for all experiments. The rats were kept in groups of 4 in cages (43 cm long, 25 cm wide, and 18.5 cm high), maintained on a 12-hour light/dark cycle, with access to food and water *ad libitum*. Animals were randomly assigned to nine groups (n = 7):

- Control group: Rats were intact in the control group.
- Ischemic group: The rats had surgery following the permanent MCAO rat model procedure.
- Sham-operated (no-ischemia + vehicle) group: The rats received saline (intraperitoneal; IP) administered 30 minutes, and 2 hours after surgery without MCAO. In this group, saline injection was continued for 7 consecutive days (once a day).
- Ischemia + vehicle group: In ischemic rats, saline was administered (IP) 30 minutes and 2 hours after a MCAO surgery. In this group, saline injection was continued for seven consecutive days (once a day).
- Groups 5, 6, and 7 (no-ischemia + ASA 20, 40, 80): Aspirin at the doses of 20, 40, and 80 mg/kg IP was administered 30 minutes and 2 hours to the rats in groups 5, 6, and 7, respectively, after surgery without performing a MCA occlusion. In these groups, Aspirin injections were continued for seven consecutive days (once a day).
- Groups 8 and 9 (ischemia + ASA 20 and 80): Aspirin at the doses of 20 and 80 mg/kg IP was administered to the rats in groups 8 and 9, respectively, 30 minutes and 2 hours after stroke onset. In these groups, aspirin injections were continued for seven consecutive days (once a day).

Equivalent volumes of saline were given to vehicle-treated rats. After taking into account 35% mortality in ischemic groups and 10% lack of neurological response related to ischemic induction, 72 rats were used in the present study. Aspirin was purchased from Sigma (UK) and prepared fresh each day by dissolving in distilled water. Animals were treated and cared for according to the ethical guidelines for research at Kerman University of Medical Sciences (Ethical code: 1385.75). All measures were taken to reduce animal suffering and the number of animals used during experimental procedures. Behavioral tests were blind and conducted during the light phase of the cycle.

Animal model of focal cerebral infarction

The rats were anesthetized with intraperitoneal ketamine (80 mg/kg) in combination with xylazine (10 mg/kg) and fixed on a stereotaxic frame (Stoelting, USA). The level of anesthesia was controlled by corneal reflex monitoring. Briefly, a skin incision was made under adequate anesthesia between the left external auditory canal and the left lateral canthus after shaving the left temporoparietal region. The temporal muscle was retracted, and a small burr hole was drilled into the temporal bone. Throughout the procedure, heat injury was prevented by applying saline to the area. After removing the inner layer of the skull, the MCA was permanently cauterized while taking care not to damage the brain surface. After occlusion of the MCA, the temporalis muscle and skin were closed in layers. Following the occlusion of the left MCA, common carotid arteries were exposed by midline anterior cervical incisions. Both common carotid arteries were then occluded with microaneurysm clips for one hour. Neurological impairment in the stroked animals was

confirmed by criteria such as turning to one side and decreasing the level of consciousness or mobility. The body temperature was maintained at 37 °C to 38 °C with a heating pad during the surgery. All behavioral tests were performed after a post-surgical recovery period of 7 days (22).

Spatial learning: MWM

The water maze was a black circular tank with a diameter and height of 1.36 and 0.6 m, respectively, filled 25 cm deep with water (20°C ± 1 °C). A 10 cm × 10 cm platform made of metal was placed in the center of the northeast quadrant ~1.0 cm below the water surface. The pool was located in a large room, and some visual cues, including reflective geometric shapes, were hung on the wall. The swimming activity of each rat was monitored by a video camera connected to a computer through an image analyzer that relayed information, including latency to find the platform, total distance traveled, time and distance spent in each quadrant, etc (23-25).

Behavioral procedures

Rats received four trials a day as part of a 4-day training session. In each trial, the animal was permitted to escape towards the hidden platform from the designated starting points (North, South, East, and West), where it was initially placed into the water facing the wall. Every starting point was used once daily, meaning each trial started from a different starting point. The rat was allowed to swim for a maximum of 90 seconds in each trial to find the platform. Whether the animal failed or found the platform, it was allowed to remain on the platform for 30 seconds. In the inter-trial interval (ITI), the animals were taken from the maze to a holding cage to rest for 30 seconds. In the probe trials on day 5, 24 hours after the last training day, the distance traveled and time spent in each quadrant were recorded and analyzed (23-25).

Statistical analysis

The test for normality of data distributions was performed with the Shapiro–Wilk test in GraphPad Prism software (La Jolla, CA). Then, the data such as path length (distance moved) and escape latency were analyzed using repeated-measures analysis of variance (RMA). When significant differences were found, the least significant difference (LSD) test was used. Contrast analysis was performed between days 1 and 4 during learning acquisition days. One or two-way analysis of variance (ANOVA) was used to compare experimental groups and probe trial data. If ANOVA indicated a significant difference, the groups were compared using the LSD post hoc test. Data are presented as mean ± SEM. *P* values less than 0.05 were statistically significant.

Results

Effects of focal ischemia of MCA on learning and memory

Spatial learning

The first experiment evaluated the effects of focal MCA occlusion on memory and learning in rats. The ability to locate the platform in all groups, control, ischemia, vehicle, and vehicle + ischemia, improved over the four days. The RMA analysis of the groups showed significant differences in swimming path length or distance moved ($F_{2.4, 59} = 37.3$, $P = 0.001$) and escape latency ($F_{2.2, 54.1} = 29.3$, $P = 0.001$) to find the platform during learning days. The average of these parameters was lower in all groups on day 4 than on day 1 ($P = 0.001$). There is not any relationship between day*group for the distance moved ($F_{7.3, 59} = 1.2$, $P = 0.31$) and escape latency ($F_{6.7, 54.1} = 0.67$, $P = 0.69$).

As Figure 1a shows, the distance moved was longer in the ischemic rats (the ischemia and the vehicle + ischemia groups) than in the control and vehicle groups. Post-hoc analysis performed by LSD showed that the distance moved increased significantly in the vehicle + ischemia group compared to the vehicle group on day 2 ($P = 0.014$), day 3 ($P = 0.027$), and day 4 ($P = 0.03$). Also, there was a significant difference between the ischemia and the control groups on day 2 ($P = 0.02$). There was no significant difference between the control and vehicle groups and between the vehicle + ischemia and the ischemia groups. The escape latency was also longer in the ischemic rats compared to the control group. Post hoc analysis performed by LSD showed that the escape latency increased significantly in the vehicle + ischemia group compared to the vehicle group on day 2 ($P = 0.048$), day 3 ($P = 0.029$), and day 4 ($P = 0.043$). Similarly, on day 2, the ischemia group showed a significant difference from the control group ($P = 0.005$). The control and vehicle groups, and also, the vehicle + ischemia and ischemia groups were not significantly different (Figure 1b).

Probe test

A two-way ANOVA for the distance moved revealed significant main effects for the ischemia effect ($F_{1, 23} = 8.1$, $P = 0.001$) but not for the injection effect, and also, the interaction between ischemia * injection. For the percentage of time in the trigger zone, two-way-ANOVA revealed significant main effects for the Ischemia effect ($F_{1, 23} = 12.1$, $P = 0.001$) but not for injection effect, and also, the interaction between Ischemia × injection. Because the main effects were significant, a one-way ANOVA was followed by LSD. This analysis showed that the percentage of distance moved in the trigger zone in the ischemic groups (ischemia and ischemia + vehicle) decreased significantly compared to the control and no-ischemia + vehicle groups ($P = 0.001$ and $P = 0.036$, respectively). The time in the trigger zone in the ischemic groups (ischemia and the ischemia + vehicle) also decreased significantly compared to the control and no-ischemia + vehicle groups ($P = 0.04$

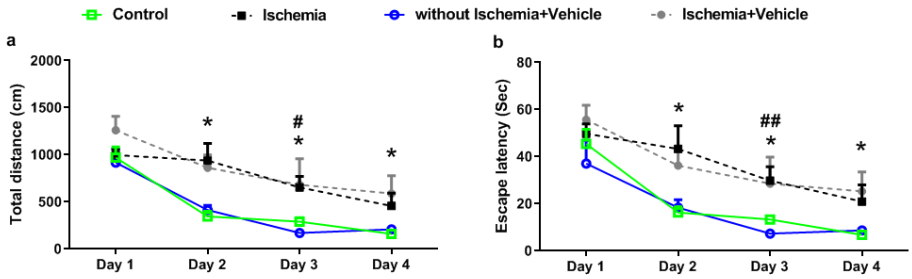

Figure 1. Performance of rats in the water maze following ischemia during learning acquisition days. Signs (*) and (#) show a significant statistical difference between total distance moved (a) and escape latency (b) between the ischemia + vehicle group vs. the vehicle group, and the ischemia group vs. the control group. Data are shown as mean ± SEM.*$P<0.05$, #$P<0.05$, and ##$P<0.01$

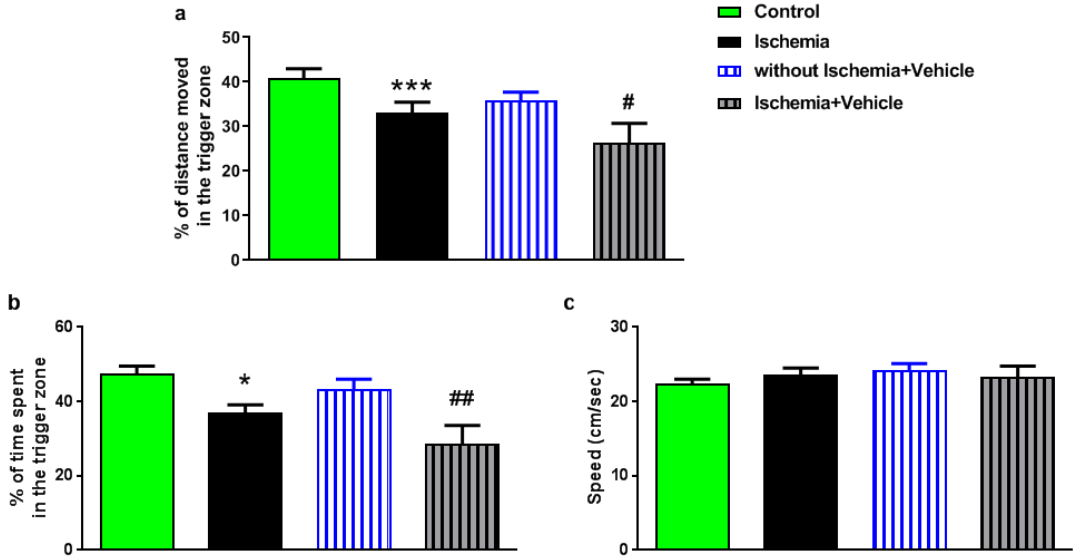

Figure 2. The percentage of distance moved (a) and the percentage of time in trigger zone (b), and swimming speed (c) of rats in the water maze following ischemia during the probe test. Signs (*) and (#) show a significant statistical difference between the ischemia + vehicle group vs. the control group and the without ischemia vehicle vs. Ischemia + vehicle, respectively. Data are shown as mean ± SEM.* $P<0.05$, ***$P<0.001$, #$P<0.05$, and ##$P<0.01$

and $P=0.006$, respectively) (Figures 2a and 2b).

Effects of repeated aspirin administration on memory and learning

Spatial learning

In the second experiment, how memory and learning are affected by repeated aspirin administration was evaluated. Aspirin, at three doses of 20, 40, and 80 mg/kg, was injected intraperitoneally 30 min and 2 hours after an operation procedure without ischemia induction in the ASA-treated rats, and the injections were continued for seven consecutive days (once a day).

Based on the first experimental results, spatial memory and learning of ASA-treated rats were compared to the vehicle group. RMA revealed that the distance moved ($F_{3, 72}=28.0$, $P=0.001$) and escape latency ($F_{3, 72}=24.6$, $P=0.001$) were significantly different during learning days. The average of these parameters was lower in all groups on day 4 than day 1 ($P=0.001$). There was no relationship between day and group for the distance moved ($F_{9, 72}=1.08$, $P=0.38$) and escape latency ($F_{9, 72}=1.28$, $P=0.25$).

As shown in Figure 3a, the distance moved was longer in the ASA-treated rats than in the control and the vehicle groups. Post-hoc analysis performed by LSD showed that the distance moved increased significantly in the ASA (20 mg/kg) group compared to the vehicle group on day 2 ($P=0.008$), day 3 ($P=0.003$), and day 4 ($P=0.01$). A significant difference was also found between the ASA-treated (40 and 80 mg/kg) groups and the vehicle group on day 2 ($P=0.008$ and $P=0.049$, respectively). There was no significant difference among the ASA-treated groups. The escape latency also escalated in the ASA-treated rats compared to the vehicle group. Post-hoc analysis performed by LSD showed that the escape latency increased significantly in the ASA (20 mg/kg) group compared to the vehicle group on days 2 ($P=0.014$), 3 ($P=0.008$), and 4 ($P=0.05$). Compared to the vehicle group, there was a significant increase in the escape latency on days 1 and 3 in the ASA (40 mg/kg) group ($P=0.05$ and $P=0.024$). No significant difference was observed between the vehicle and ASA (80 mg/kg) groups during the learning days. The ASA-treated groups

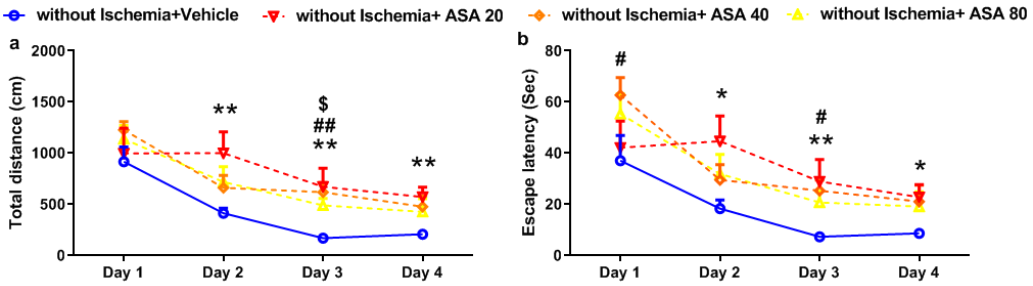

Figure 3. Performance of rats without induced ischemia following administration of saline (vehicle) or aspirin (ASA) with different doses (20, 40, and 80 mg/kg) during learning acquisition days in the water maze. Locations of significant statistical differences for the total distance moved (a) and escape latency (b) are shown as a sign (*), (#), and ($) for the ASA-treated rats at doses of 20, 40, and 80 mg/kg vs. the vehicle group, respectively. Data are shown as mean ± SEM.*$P<0.05$, $^{\$}P<0.05$, $\#P<0.05$, and $\#\#P<0.01$.

showed no significant difference either (Figure 3b).

Probe test

A one-way ANOVA followed by LSD showed there was a significant main effect for the percentage of distance moved ($F_{3, 24}=9.06$, $P=0.001$) and percentage of time in the trigger zone ($F_{3, 24}=11.4$, $P=0.001$) but not for swimming speed ($F_{3, 24}=1.31$, $P=0.29$) during the probe trial. As shown in Figures 4a and 4b, the percentage of distance moved in the trigger zone in the ASA-treated groups that were not ischemic (the no-ischemia + ASA 20, 40, and 80 mg/kg) decreased significantly compared to the no-ischemia + vehicle group ($P=0.001$). Also, the percentage of time in the trigger zone in these ASA-treated groups decreased significantly compared to the no-ischemia + vehicle group ($P=0.001$).

Effects of repeated aspirin administration on impaired memory and learning following focal MCA occlusion

Spatial learning

Repeated aspirin administration's effects on impaired memory and learning were evaluated after MCA occlusion. Because there was no significant difference among the ASA-treated groups, just low and high doses of aspirin were considered and investigated in the third set of experiments. Aspirin at doses 20 and 80 mg/kg was injected intraperitoneally for 30 minutes and 2 hours in the ischemic rats, and injections were continued for seven consecutive days (once a day).

RMA revealed a significant difference during the learning days in the distance moved ($F_{2.7, 66.4}=27.4$, $P=0.001$) and escape latency ($F_{26.3, 63.1}=21.9$, $P=0.001$). The average of these parameters was lower in all groups on day 4 than on day 1 ($P=0.001$). There is no relationship between day and group for the distance moved ($F_{8.3, 66.4}=0.38$, $P=0.92$) and escape latency ($F_{7.78, 63.1}=0.09$, $P=0.99$).

As shown in Figure 5a, the distance moved was longer in the ASA 80 mg/kg + ischemia group compared to the vehicle group Days 1 ($P=0.02$), 2 ($P=0.01$), and 3 ($P=0.001$). The distance moved decreased significantly in the ASA 20 mg/kg + ischemia group compared to the ASA 80 mg/kg + ischemia group on day 1 ($P=0.016$), day 2 ($P=0.005$), and day 4 ($P=0.014$). Although the ASA 20 mg/kg + ischemia group and the vehicle + ischemia group were not significantly different, the rats in the ASA 20 mg/kg + ischemia group moved a shorter distance than the vehicle + ischemia group. They behaved similarly to the vehicle group on days 1 and 2.

As shown in Figure 5b, the escape latency was longer in the ASA 80 mg/kg + ischemia group than in the vehicle group on days 1 ($P=0.005$), 2 ($P=0.02$), 3 ($P=0.008$), and 4 ($P=001$). In addition, the escape latency increased in the ASA 80 mg/kg + ischemia group compared to the vehicle + ischemia group on day 4 ($P=0.05$). The escape latency decreased significantly in the ASA 20 mg/kg + ischemia group compared to the ASA 80 mg/kg + ischemia group on Days 1 ($P=0.04$), 2 ($P=0.024$), and 4 ($P=0.013$). It has been noted that there was no statistically significant difference between the vehicle + ischemic group or vehicle group and the ASA 20 mg/kg + ischemia group. The rats in the ASA 20 mg/kg + ischemia group had lower escape latency than those in the vehicle + ischemic group (Figure 5b).

Probe test

A one-way ANOVA followed by LSD showed that there was a significant main effect for the percentage of distance moved ($F_{3, 24}=2.5$, $P=0.05$) and percentage of time in the trigger zone ($F_{3, 24}=3.4$, $P=0.033$) but not for swimming speed ($F_{3, 24}=0.29$, $P=0.83$) during the probe trial. The percentage of distance moved in the trigger zone in the ASA-treated groups with an ischemic (the ischemia + ASA 20 and 80 mg/kg) experience did not significantly differ from the no-ischemia + vehicle group. Also, the percentage of time in the trigger zone in these ASA-treated groups did not significantly differ from the no-ischemia + vehicle group (Figure 6a and 6b). It has been noted that the percentage of distance moved and time in the trigger zone in the ischemia + vehicle showed a significant decrease compared to the no-ischemia + vehicle group ($P<0.05$).

Discussion

Effects of focal ischemia of MCA on learning and memory

The ischemic brain damage pattern in many ischemic stroke patients can be reproduced by MCAO in focal

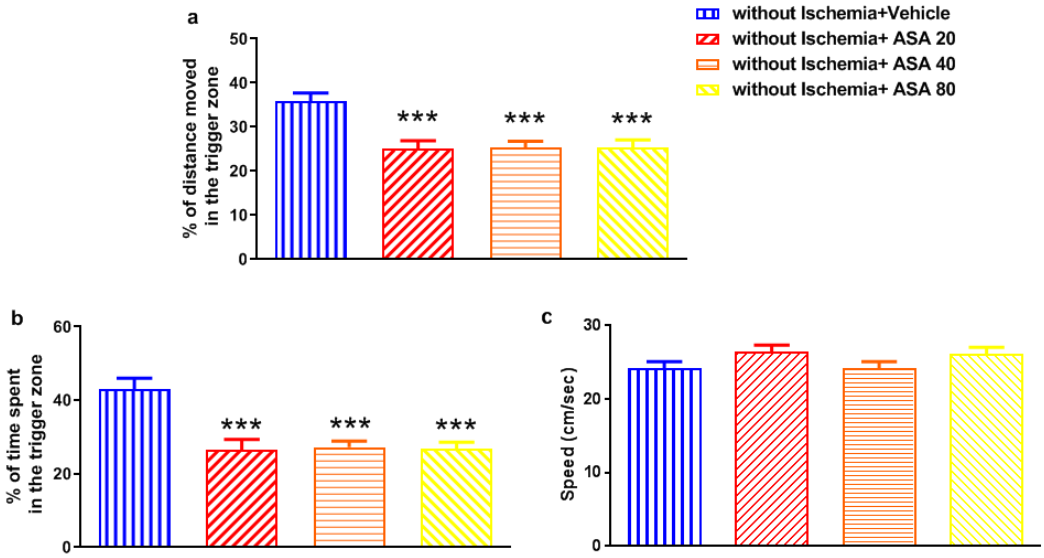

Figure 4. Performance of distance moved (a) and the percentage of time in trigger zone (b), and swimming speed (c) of rats in the water maze without induced ischemia following administration of saline (vehicle) or aspirin (ASA) with different doses (20, 40, and 80 mg/kg) during the probe test. Locations of significant statistical differences are shown as a sign (*) for the ASA-treated rats at doses of 20, 40, and 80 mg/kg vs. the vehicle group, respectively. Data are shown as mean ± SEM. ***$P<0.001$

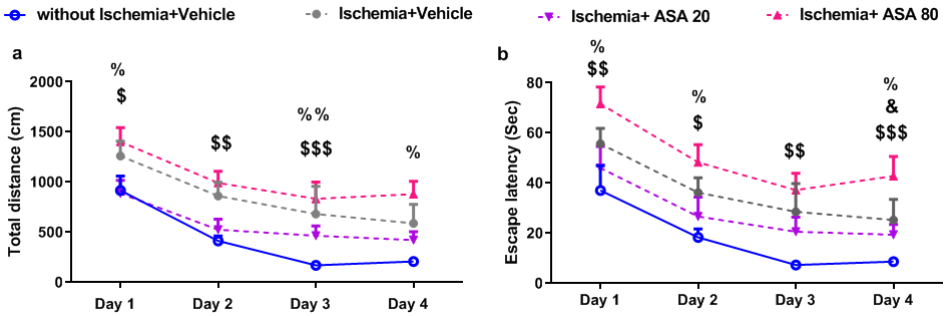

Figure 5. Performance of rats with (without) induced ischemia following administration of saline (vehicle) or aspirin (ASA) with different doses (20 and 80 mg/kg) during learning acquisition days in the water maze. Locations of significant statistical differences for the total distance moved (a) and escape latency (b) are shown as a sign (&), ($), and (%) for the ASA 80 mg/kg group vs. the Ischemia + vehicle group, the ASA 80 mg/kg group vs. the without Ischemia + vehicle group, and the ASA 80 mg/kg group vs. the ASA 20 mg/kg group, respectively. Data are shown as mean ± SEM. $^{\$}P<0.05$, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$, $^{\%}P<0.05$, $^{\%\%}P<0.01$, and $^{\&}P<0.01$.

cerebral ischemia animal models. Focal ischemia can be induced by permanently or temporarily blocking the MCAO, producing a reliable and reproducible model of choice for focal ischemia studies (26,27). Ischemic stroke is commonly followed by memory impairment (28). The present study found that focal ischemia negatively impacts the learning and spatial memory of MCAO-treated Wistar rats. In a study that used MCAO-induced stroke rats, learning and memory were significantly impaired, as indicated by the passive avoidance task. The MCA occlusion of the left and right hemispheres can also lead to sensorimotor impairment and increase anxiety levels in animals (29). In a previous study, the spatial memory of MCAO-treated mice assessed by a novel object recognition test showed a significant reduction of the MCAO-treated mice's preference for the novel object during the test phase compared to intact and sham-operated groups. This study assessed the morphometric parameters of cortical neurons on day 60 of the post-ischemic period using the hematoxylin-eosin method. Histological analysis showed that cortical cells in the ischemia group displayed significant structural alterations, with approximately 10% of these neurons exhibiting increased diameters and deformed contours, including a high incidence of hyperchromic and polygonal neurons. Additionally, structural changes in the brain matter were observed (30). Moreover, in another study, the results of the passive avoidance test showed that acquisition, reacquisition, and retention of memory were notably disturbed in the MCA-occluded rats (31).

Clinical studies found an increased risk of cognitive and memory impairment after a stroke; the Mini-Mental State Examination (MMSE) was used to evaluate the cognitive performance, indicating a significant difference in post-stroke patients than the normal control group (32).

In the study by Fernades et al, after permanent MCAO in mice, open field, Y-maze, novel object recognition, and MWM test indicated that there was a remarkable decrease

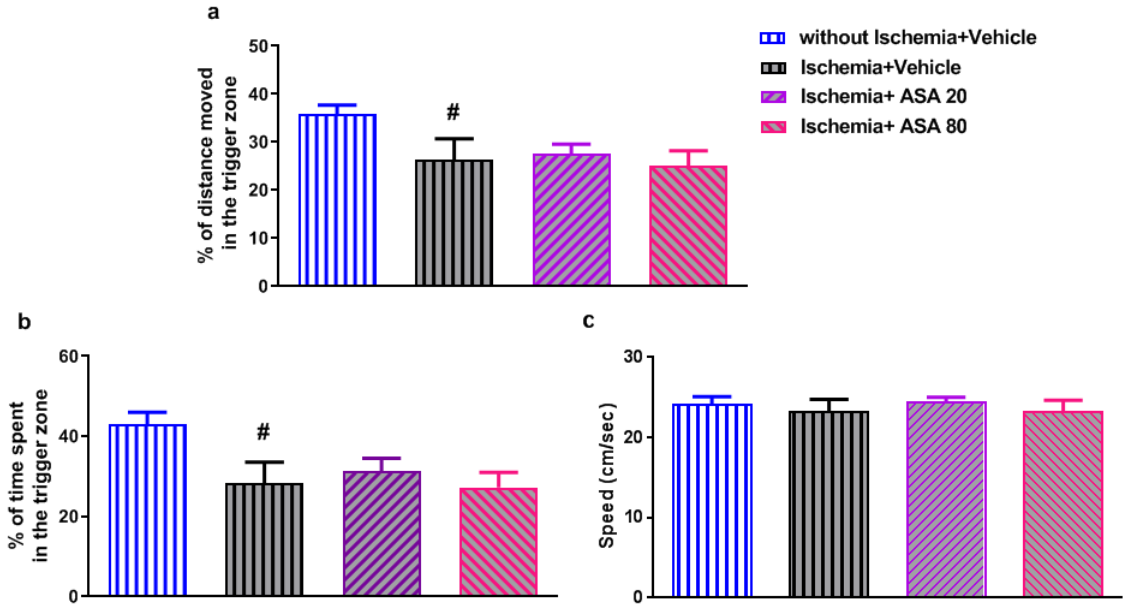

Figure 6. Performance of distance moved (a) and the percentage of time in trigger zone (b), and swimming speed (c) of rats in the water maze following administration of saline (vehicle) or aspirin (ASA) with different doses (20 and 80 mg/kg) during probe test in the water maze. Significant statistical differences are shown as a sign (#) for the without Ischemia + vehicle group vs. Ischemia + vehicle group. Data are shown as mean ± SEM. #$P < 0.05$.

in the short- and long-term working, recognition, and spatial memories of the MCAO-treated group compared to normal rats. Our water-maze test data were consistent with their study and showed an increase in distance moved and escape latency to find the platform during learning days, also confirmed by the probe test, indicating the negative impact of stroke on spatial memory (33). The reason for the effects of ischemia on memory and cognitive dysfunction is inflammation, acidosis, excitotoxicity, oxidative stress, and other contributing factors, which lead to the death of neurons responsible for the storage and reproduction of information. The contributing factors could be disruptions in the synaptic plasticity processes and circuitry after ischemia, resulting in abnormal neural dynamics within different brain networks that play an important role in memory processing (34-36). Furthermore, it is also shown that the population of neurons in the medial striatum, involved in the processes of remembrance of the exact location of objects (navigation), is significantly reduced after a 30-minute episode of brain ischemia (37).

It has been shown that when the left and right common carotid arteries are temporarily ligated, the percentage of normal cells in the CA1, CA2, CA3, and CA4 regions of the hippocampus markedly decreases (38). Following MCAO, both local blood flow and local glucose appear to decrease. Moreover, neurons in this area develop hypoglycemia. These conditions block the reuptake of glutamate, increasing its extracellular levels. The role glutamate plays in the pathogenesis of cerebral ischemia is well-established (39). Glutamate is an essential excitatory neurotransmitter activating NMDA receptors in the hippocampus. Although glutamate is effective in memory and learning (39,40), its toxic increase in extracellular fluid following MCAO can increase cellular calcium and activate apoptosis (41,42). Permanent occlusion of the MCA causes infarction in the subcortical region. There are also signs of ischemia in the striatum, septum, thalamus, and hippocampus. Infarct areas with pancellular necrosis, eosinophil region, and wrinkled neurons have been detected along the edge of the infarct area (43). We observed previously the degenerated pyramidal neurons in the CA1 region of the hippocampus well after the MCAO. Consequently, the present study confirmed the negative effect of focal ischemia on learning and memory, as shown in previous studies (15,44).

Effects of repeated administration of aspirin on learning and memory

Depending on its dosage, acetylsalicylic acid (aspirin) has a broad range of pharmacological properties, such as antiplatelet, analgesic, and anti-inflammatory effects, via multiple pharmacological mechanisms (45,46). It is not clear precisely how cognitive function is affected by NSAIDs, including aspirin. If the neuronal function is affected by a specific inflammatory condition, NSAIDs can be used to improve the adverse condition.

The opinion that aspirin's effectiveness in stroke treatment depends on pathways beyond its antiplatelet function is supported by increasing evidence. Almost none of the studies investigating the ameliorative effect of aspirin on focal cerebral ischemia rat learning and memory, specifically spatial memory, have reported any significant difference between control, sham, or aspirin-treated normal rat groups (10,47-50). The present study indicates that the administration of aspirin at doses of

20, 40, and 80 mg/kg can reduce the learning and spatial memory of normal rats in the MWM test. Unlike the previous studies, our results indicated that despite its multiple clinical benefits, aspirin could also negatively affect learning and spatial memory. Since aspirin is a widely used over-the-counter drug, our findings showed the need for further studies to understand the exact impact of aspirin on learning and memory. In this study, three doses of aspirin (20, 40, and 80 mg/kg) were investigated. As the behavioral effect of aspirin 40 mg/kg was similar to that of the 80 mg/kg doses, we selected 20 and 80 mg/kg for the next part of the study.

Effects of repeated ASP administration on impaired memory and learning following focal MCA occlusion

The main finding of this study is that a modest postoperative treatment regimen with a low dose of aspirin has a protective effect on cognitive decline after MCAO. However, aspirin had no ameliorating effect on memory and learning at high doses. During the testing period, a progressive improvement was noted in task learning in all experimental groups; however, significant impairment of spatial learning scores was caused by aspirin in non-ischemic rats compared to the control group. Moreover, measurements of swimming speed among different groups in the present study suggest that the observed learning improvements were not caused by limb flexibility or motor output. In this regard, in an original study, it was shown that there was a significant improvement in spatial learning of ischemic rats after aspirin administration; however, the swim speed difference was not significant (44). The protective effect of aspirin at low doses on dementia and different subtypes of cognitive impairment was supported by Li and colleagues' study, an investigation of 12 cohort studies (51).

The previous study also showed that by preventing the decrease in ATP levels induced by ischemia, the accumulation of glutamate was reduced by aspirin at a dose of 30 mg/kg 2 hours before ischemia induction in the permanent MCAO rat model during the early stages of cerebral ischemia (39,52). Aspirin treatment is associated with significantly lower glutamate concentrations in the CSF after acute cerebral infarction in humans (52,53). Furthermore, a low dose of aspirin can act as an antiplatelet agent, promoting cerebral blood flow in the memory and cognitive areas after stroke, and protecting cognitive function and memory (54). In addition, by stimulating alternative microglia activation and mitigating the neurotoxicity, lipoxins triggered by aspirin can reduce the impact of neuroinflammation on cerebral small vessel disease (55). At high doses used in the present study, the neurotoxic effects of aspirin may prevent cognitive dysfunction improvement. At low doses, various mechanisms are involved. However, due to MCA cauterization, reduced blood flow to this area of the brain undermines these suggested mechanisms.

Apart from the previously mentioned effect, the neuroprotective properties of can be improved by other mechanisms as well. Previous studies have demonstrated that NMDA receptor activation by the glutamate released following an ischemic injury plays a role in inducible nitric oxide synthase (iNOS) enzyme expression. Thus, glutamate release inhibition may increase neuroprotective effects by inhibiting the delayed expression of inflammatory enzymes such as iNOS or cyclooxygenase type II (COX-2) (56). Some of the neuroprotective qualities of aspirin have been attributed to activities such as oxidative stress or NF-κB inhibition; however, these effects only occur at very high concentrations, correlating with anti-inflammatory dosage (57,58).

While ASA doses as low as 0.5 mg/kg inhibit COX-1 and platelet aggregation (59, 60), doses as high as 30 mg/kg are necessary for COX-2 inhibition and full anti-inflammatory effect (45,61). However, how aspirin and other NSAIDs positively affect infarction has not yet been fully explained. If it is a specific inflammatory condition affecting neuron function, NSAIDs can help reduce these factors. It seems unacceptable to attribute the effects of aspirin on ischemic injury only to increased local blood flow. It has long been mentioned that aspirin has antithrombotic properties and prevents platelet aggregation (62). It also helps reduce inflammation by blocking COX, an enzyme that helps form prostaglandins and ultimately "inflammation, swelling, pain and fever" (59,62).

Conclusion

In summary, though it is not fully known how aspirin reduces ischemic injury after MCAO, cognitive function can be improved by multiphase administration of a relatively low (20 mg/kg) aspirin dose early after stroke onset and on subsequent days. This study demonstrated the importance of administering the appropriate dosage of aspirin to mitigate adverse cognitive effects while optimizing its benefits. A monitoring system can be established to track aspirin use and its cognitive impacts, aiding policy decisions and identifying trends. Additionally, educational initiatives are needed to raise awareness among healthcare professionals and the public about aspirin's potential cognitive effects, including associated risks and benefits. However, further research is necessary to understand the mechanisms behind aspirin's conflicting effects on cognitive function to guide future policy decisions. It is suggested that future studies investigate how early aspirin should be administered after ischemia, how long the treatment should continue, and how many doses should be administered in that time for the treatment to be beneficial.

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Authors' Contribution

Conceptualization: Vahid Sheibani, Mohammad Reza Afarinesh, Ali Shamsara.
Data curation: Mandana Jafari, Zahra Hajializadeh.
Formal analysis: Mohammad Reza Afarinesh, Narges Ashraf Ganjooei.
Funding acquisition: Narges Ashraf Ganjooei.
Investigation: Narges Ashraf Ganjooei.
Methodology: Vahid Sheibani, Ali Shamsara.
Project administration: Narges Ashraf Ganjooei, Vahid Sheibani.
Resources: Narges Ashraf Ganjooei.
Software: Mohammad Reza Afarinesh.
Supervision: Vahid Sheibani, Mohammad Reza Afarinesh.
Validation: Ali Dehghani.
Visualization: Vahid Sheibani.
Writing–original draft: Narges Ashraf Ganjooei, Mohammad Reza Afarinesh.

Competing Interests

The authors declared no potential conflicts with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was approved by the local medical ethics committee for the use and care of animals at Kerman University of Medical Sciences (Ethical Code: EC/ KNRC/83/32).

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