Research Article

Remote ischemic preconditioning combined with atorvastatin improves memory after global cerebral ischemia-reperfusion in male rats

Azim Hedayatpour[1], Maryam Shiasi[1], Peyman Modarresi[2], Alieh Bashghareh[1]

1 *Department of Anatomy, Tehran University of Medical Sciences, School of Medicine, Poursina St., Enghelab Ave., Tehran 1417653761, Iran*
2 *School of Veterinary Medicine, Azad University St, Shabestar, East Azerbaijan Province 5381637181, Iran*

Corresponding author: Alieh Bashghareh (alieh.bashghareh@yahoo.com)

Abstract

Introduction: Damage to hippocampus can occur through ischemia. Memory problems are among the most significant disabilities after stroke. Therefore, improving memory is of great interest in helping post-stroke patients. This study demonstrated that intraperitoneally injection of atorvastatin with a short cycle of ischemia-reperfusion in the left femoral artery improved hippocampal CA1 neurons injury and memory problems after global cerebral ischemia.

Materials and methods: In this article survey, we used 64 animals. Rats were divided into 8 groups, (n=8). Group 1: control; group 2: sham; group 3: global cerebral ischemia (GCI) only; group 4: remote ischemic preconditioning (RIP) + GCI; group 5: GCI + atorvastatin (ATO); group 6: GCI + vehicle; group 7: RIP + GCI + ATO; group 8: RIP + GCI + vehicle. We created global cerebral ischemia (GCI) with 20 min occlusion of the Common carotid artery.

Results and discussion: Remote ischemic preconditioning could improve rats performance in water maze tests along with a decrease in neuronal death. Also, atorvastatin combined with remote ischemic preconditioning was more effective for memory improvement and reduction of neuronal death. Inconsistent with our result, the function of the animals in the ischemia group was impaired. CA1 hippocampal neurons have an important role in memory and learning, and they can be damaged after cerebral ischemia. Therefore, ischemia can create memory problems. Remote ischemic preconditioning and atorvastatin had a neuroprotective effect and could improve rat performance in water maze test.

Conclusion: This study showed that remote ischemic preconditioning with atorvastatin could improve CA1 neuronal injury and memory.

Graphical abstract:
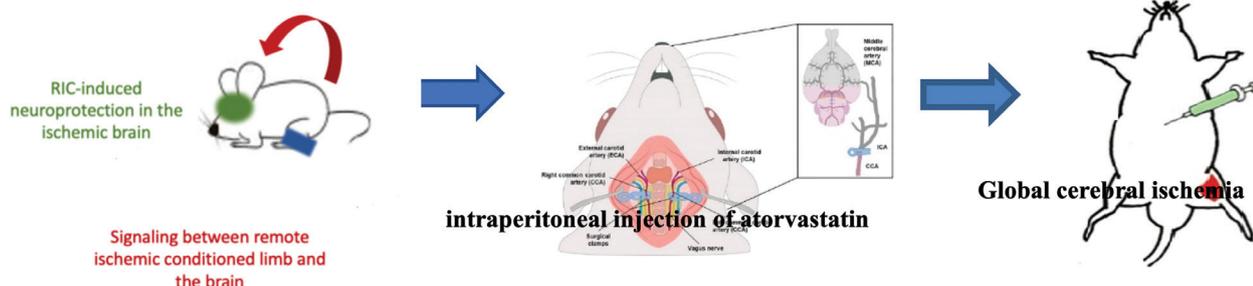
Keywords

Morris water maze, ischemia, remote ischemic preconditioning, atorvastatin, memory.

Introduction

Stroke not only causes physical disorders, but also cognitive problems (Farovik et al. 2010; Godinho et al. 2018; Lugtmeijer et al. 2021). The pyramidal CA1 neurons of the hippocampus have an important role in spatial learning and memory (Vorhees and Williams 2006; Farovik et al. 2010). It has been demonstrated that ischemia reduces the number of hippocampal neurons and produces memory problems demonstrated in various behavioral tests. Therefore hippocampal damage following ischemia can create memory deficit (Vorhees and Williams 2006; Ólafsdóttir et al. 2018). Statins, one of the common group of drugs, reduce the synthesis of cholesterol and are used for high cholesterol patients. Atorvastatin is a member of the family of statins often used in hypercholesterolemia treatment (Veighey and MacAllister 2012). Studies have shown atorvastatin (ATO), both in pretreatment and post-treatment, can decrease hippocampal neurons death (Berger et al. 2008; Hu et al. 2013; Ren et al. 2021). Statins cause angiogenesis in ischemic limbs (Ding et al. 2012). Furthermore, they have a neuroprotective effect with activating phosphatidylinositol-3 kinases (PI3K) AKT signaling pathway, which results in cell proliferation, differentiation, and migration (Kesner et al. 2010). Remote ischemic preconditioning (RIP) involves short cycles of ischemia-reperfusion (Hu et al. 2013). Remote ischemic preconditioning is one of the strategies that improve neuronal injury following global cerebral ischemia (Pérez-Pinzón 2004; Wang et al. 2016). According to some studies, remote ischemic preconditioning has a neuroprotective effect on CA1 neurons and memory after cerebral ischemia (León-Moreno et al. 2020). The goal of cerebral ischemia (focal and global) models is to create an animal model for studying the consequence of ischemia. Transient occlusion of the bilateral carotid arteries is often used as animal models for inducing global cerebral ischemia. Following stroke, memory problems accrued, and pretreatment strategies can help the ischemic patient. Statins are used as a treatment for ischemia patients or as pretreatment for high-risk people. Atorvastatin is a statin that has a neuroprotective effect (Mounier et al. 2021). Remote ischemic preconditioning is a pretreatment strategy that can improve neuronal injury against ischemia (Bachevalier and Meunier 1996). Therefore, we wanted to study two effective treatments together to earn reduce the neuronal injury after global cerebral ischemia. On the other hand, we designed this study to determine the role of remote ischemic preconditioning combined with atorvastatin in neuronal damage in the hippocampus and memory function following global

cerebral ischemia. CA1 neuronal injury has been shown with the Nissl assay. Memory was examined using a water maze; the Morris water maze is one of the credible tests for investigating psychological processes and neural mechanisms of spatial and working memory (Bachevalier and Meunier 1996).

Materials and methods

Animal care

Temperature (22±1 °C) and humidity (40–70%) were controlled, and the cages were with a 12 h light/dark cycle (lights on at 7:00 A.M.). All behavioral tests were performed throughout the day and in the experimental room. We had accustomed all the animals to the laboratory conditions of Tehran University of Medical Sciences at least 7 days before the start of the experiment, and all the experiments were based on supervised by the Animal Ethics Committee (IR.TUMS.MEDICINE.REC.1396. 3693, 20-5-2017) of Tehran University of Medical Sciences. Male Sprague-Dawley rats weighing between 200–250 g were selected. We used 8 rats in every group.

Animal preparation and administration

Rats were in 8 groups:

Group 1: Control (n=8): neither drug nor surgical intervention was applied.

Group 2: Sham group (n=8): the rats were subjected to the same surgical way, but without occluding a common carotid artery.

Group 3: GCI group (n=8): surgery was undertaken with general anesthesia, and rats were exposed to 20 minutes of global cerebral ischemia.

Group 4: RIP + GCI group (n=8): the rats were subjected to 5-minute ischemia and 5-minute reperfusion 5 times in the left femoral artery, and after 24 h rats were exposed to global cerebral ischemia for 20 minutes.

Group 5: GCI + ATO group (n=8): surgery was undertaken with general anesthesia, and rats were subjected to 20 min of global ischemia. Then, 24 h after ischemia rats received an intraperitoneal injection of atorvastatin for 3 days.

Group 6: GCI + vehicle group (n=8): rats subjected to 20 min of global ischemia. 24 h after ischemia rats received an intraperitoneal injection of saline for 3 days.

Group 7: RIP + GCI + ATO group (n=8): rats were subjected to 5 series of ischemia-reperfusion (each cycle lasted 5 min) in the left femoral artery, and, after 24 h, rats endured global ischemia for 20 min. Then, 24 h after

ischemia rats received an intraperitoneal injection of atorvastatin for 3 days.

Group 8: RIP + GCI + vehicle group (n=8): rats were subjected to 5-minute ischemia and 5-minute reperfusion 5 times in the left femoral artery, and after 24 h, rats endured global ischemia for 20 min. Then 24 h after ischemia, rats received an intraperitoneal injection of saline for 3 days.

Remote ischemic preconditioning

Rats were anesthetized with Ketamine (100 mg/kg) and Xylazine (10 mg/kg), and then therats were subjected to 5-minute ischemia and 5-minute reperfusion 5 times by tourniquet application on the left femoral artery. During the cycles, we checked skin color and temperature (in the first phase of the experiment) (Fig. 1).
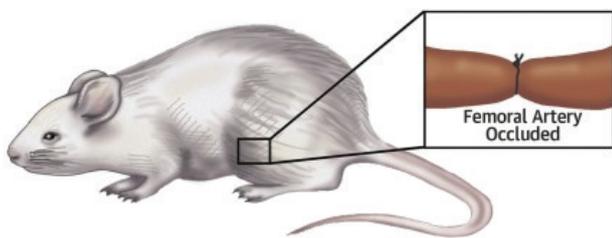

Figure 1. The animals endure 5-minute ischemia and 5-minute reperfusion 5 times on the left femoral artery to induce remote ischemic preconditioning.

Global ischemia

Firstly rats were anesthetized with an intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (10 mg/kg), and then global ischemia was induced with occluding the bilateral common carotid artery for 20 min. (Fig. 2).
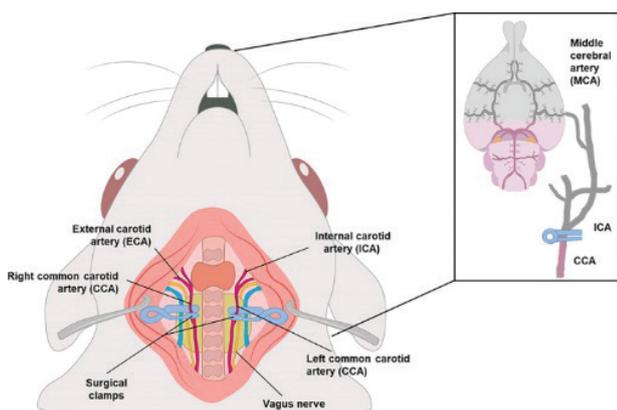

Figure 2. To create a global cerebral ischemia model, bilateral carotid arteries were occluded for 20 minutes.

Drug treatment

Atorvastatin (sigma co) that dissolved in saline was injected intraperitoneally for 3 days at a dose of 10 mg/kg 24 h after GCI. Saline was used as a vehicle. Rats were treated with atorvastatin 24 h after global cerebral ischemia for 3 days in treatment groups

Nissl staining

In this study, we needed used the brain of rats for investigating the CA1 region of the hippocampus. Seven days after treatment of rats with remote ischemic preconditioning and atorvastatin, intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (10 mg/kg) were used for rats' anesthesia, and then the animals were sacrificed. At first, rats thorax was opened and perfused with 120 ccs normal saline, and then, with 120 ccs paraformaldehyde 4% via intracranial injection. Hippocampal tissues were put in paraffin after fixation. Slides were sectioned at 5 μm thicknesses with 120 μm intervals and then were stained with 0.5% cresyl violet solution for 2 min, and finally mounted. Then sections were investigated with an Olympus microscope (CX31, Tokyo, Japan) at 400× magnification and photographed with a digital camera (Leica, München, Germany). A total number of 100 Purple-stained cells were counted as viable cells.

Morris water maze

The water maze contains a wide black tank (diameter 183 cm) filled with water (27 °C). There is an escape platform (diameter 10 cm) submerged 2 cm below the surface of the water in the southeast quadrant of the maze and the rats were to look for the platform on the trial days. During the probe test, the location of the platform could be changed to under the maze. For the visible platform task, the platform was placed 2 cm above the water surface. A large red geometric-shape tank of white texture that entirely enclosed the maze provided an extra-maze visual indication. There is a camera above the maze to monitor the rats' performance, which is connected to a computerized tracking system. In this test, the trial lasted for 3 days, and on the 4th day, a probe test was performed.

Visible test

A visible test was done to investigate the spatial learning and memory of the rats after ischemia. We indicated the location of the platform with a marker above the water, and the normal animals used the spatial cues to escape from the water. This simple related non-spatial task is considered to be independent of the hippocampus function.

Spatial acquisition test phase as spatial working memory (SAT)

In these tests, the animals were taught to spot an invisible platform by the following finding extra-maze signs. A platform (10×10 cm) was under the surface of the water (0.5 cm) in the southeast quadrant of the tank. In those tests, there were used 16 spatial trials, with the fixed start area. For every trial, the rats were allowed a 60 sec time to find the platform. If the rats failed to find the platform within 60 sec, we located the rats on the platform. There was a 20 sec break between trials. We recorded

the swimming time (sec), distance (cm), and swim speed (sec/cm). the animals with lower values had better performance, although, faster-swimming speed demonstrated better performance.

Spatial probe trial

To investigate how the rats would find the platform, one probe trial was lasted 60 sec. During the trial, the rats were supposed to find the hidden platform within 60 sec. The higher numbers for time and distance in the fourth quadrant show better performance in contrast to swim speed in the fourth quadrant. The platform in the fourth quadrant in the probe trial was removed. It helped to investigate reference memory (Vorhees and Williams 2006).

Statistics

One-way ANOVA was used for a statistical analysis, and, for more than two groups ,Turkey's test was used. In the case of comparison of two different groups, an unpaired Student t-test was used. Graph Pad Prism (version 5.0, Graph Pad Software Inc., San Diego, California) was used for statistical analyses and $p<0.05$ was considered as significant. Data were shown as mean ± SEM.

Results

Nissl assay

Our findings in the Nissl assay showed that the number of hippocampal CA1 neurons decreased after ischemia (Fig. 3b). The number of normal hippocampal CA1 neurons in the control group was 57±4, and 5±2 in the ischemia group (Fig. 3a). In treatment groups (RIP + GCI), (GCI + ATO), and RIP + GCI + ATO, the number of normal neurons significantly increased. The numbers of normal cells in RIP + GCI, and GCI + ATO groups were (30±4) and (35±3), respectively. The highest number of damaged cells was in the ischemia group, and treatment with atorvastatin and remote ischemic preconditioning could improve cells after ischemia. Furthermore, the combination use of remote ischemic preconditioning and atorvastatin can significantly improve CA 1 hippocampal neurons injury and increase the number of normal cells (42±3) in comparison with the other treatment groups (RIP + GCI), (GCI + ATO).

Water maze

Firstly, all six groups could swim easily. However, the rats in groups (GCI) and (GCI + VEHICLE) could not find the hidden platforms immediately on the training
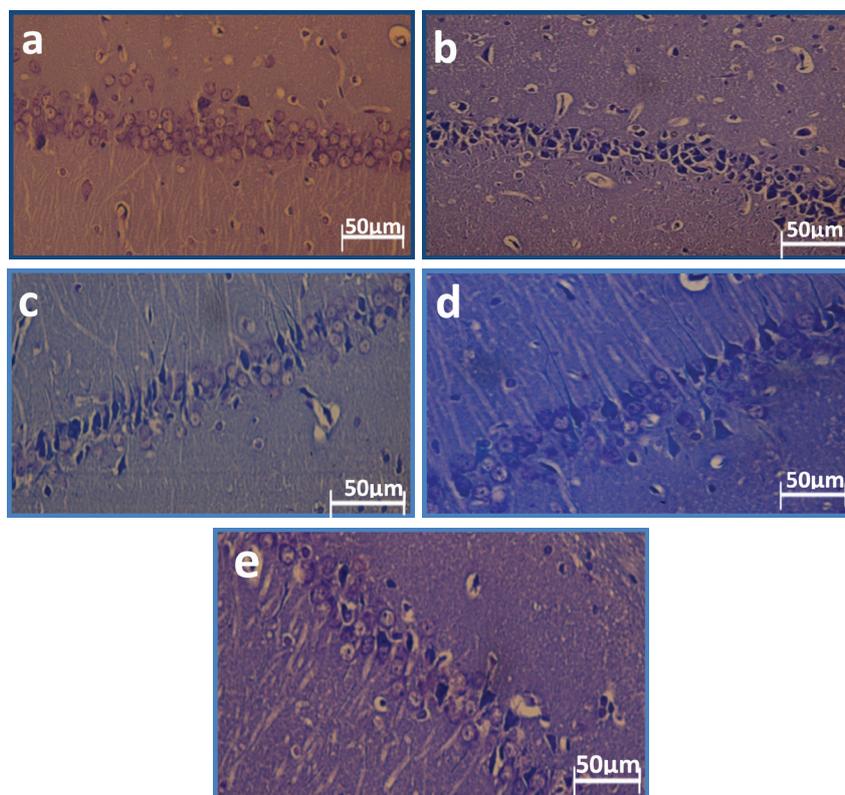

Figure 3. CA1 hippocampus neurons after 20 min of global cerebral ischemia (GCI). **a**. Control group; **b**. Ischemia group; **c**. Global cerebral ischemia (GCI) + atorvastatin (ATO) group; **d.** Remote ischemic (RIP) + global cerebral ischemia (GCI) group; **e.** Remote ischemic (RIP) + global cerebral ischemia (GCI) + atorvastatin (ATO) group. Normal cells in the control group had defined shape and range, but after ischemia the shape of neurons was destroyed.

day (Fig. 4). Remote ischemic preconditioning and atorvastatin could improve the rats' performance in a water maze test and decreased the time of finding the platform. Much less time was used by the group (RIP + GCI + ATO). In other words, using remote ischemic preconditioning and atorvastatin together could decrease the time of swimming (Fig. 4). The rats in the group (GCI) and group (GCI + VEHICLE) had less speed than the rats in the (CONTROL), (SHAME), (RIP + GCI), (GCI + ATO), (RIP + GCI + ATO), (RIP + GCI + VEHICLE), and speed significantly increased in (CONTROL), (SHAME), (RIP + GCI), (GCI + ATO), (RIP + GCI + ATO), (RIP + GCI + VEHICLE) compared to that in (GCI) and (GCI + VEHICLE). Also, the rats which had received the combined treatment with atorvastatin and RIP showed a higher speed of swimming in comparison with that in RIP + GCI and GCI + ATO groups (Fig. 5). In the probe experiment, the swimming time of the mice in the northern quarter and the number of platform intersections were used to count the maintenance performance (Fig. 6). Our findings in the probe test showed that distance in the treatment groups (RIP + GCI) and (GCI + ATO) increased, and the rats in the combined treatment group (RIP + GCI + ATO) had better performance in the maze test in comparison with other treatment groups (RIP + GCI) and (GCI + ATO).
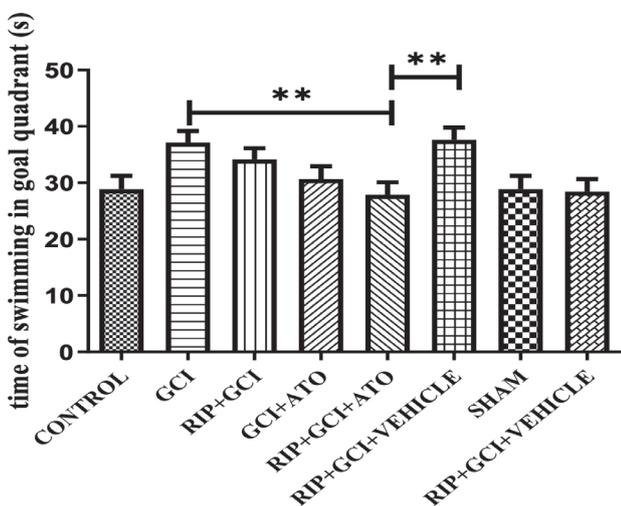
Spatial task acquisition

Figure 4. Effects of global cerebral ischemia on memory. After ischemia, time of finding the escape platform increased. Data were represented as mean±SEM, and p<0.05 was considered significant. ** was used for showing a significant difference between groups. Treatment with atorvastatin and remote ischemic decreased the time for reaching the escape platform, and the shortest time in the control and shame groups was for the combined treatment group (RIP + GCI + ATO). GCI – global cerebral ischemia, RIP – remote ischemic preconditioning, ATO – atorvastatin.
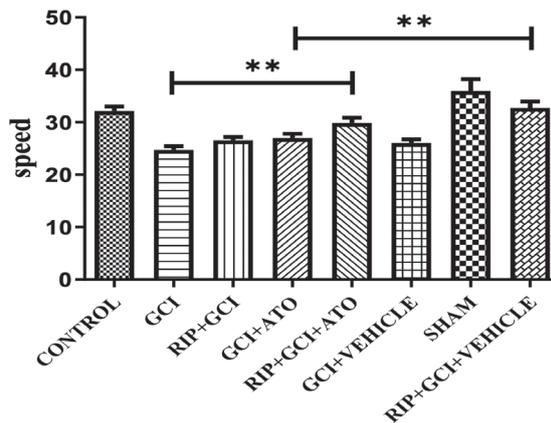

Figure 5. Effects of global cerebral ischemia on memory. Data were represented as mean±SEM and p<0.05 was considered significant. ** was used for showing a significant difference between groups. Ischemia decreased the swimming speed. The swimming speed was higher in RIP + GCI + ATO rats compared to that in other treatment groups. GCI – global cerebral ischemia, RIP – remote ischemic preconditioning; ATO – atorvastatin.
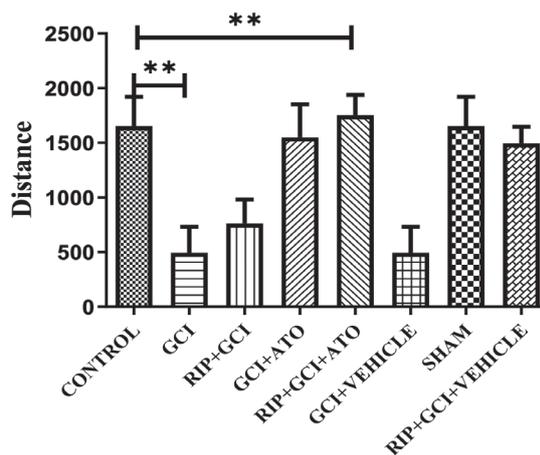
Probe test

Figure 6. Effects of global cerebral ischemia on memory. Data were represented as mean±SEM, and p<0.05 was considered significant. ** was used for showing a significant difference between groups. The swim distance decreased in the ischemia group compared to that in the other groups. The treatment groups could increase distance, and the longest distance was covered by the combined treatment group (RIP + GCI + ATO). GCI – global cerebral ischemia, RIP – remote ischemic preconditioning; ATO – atorvastatin.

Discussion

In this study, global cerebral ischemia and reperfusion cause significant cerebral injury in male mice. Ischemia in the brain causes neuronal loss in the neocortex, hippocampus, and striatum and leads to memory deficit (Lee et al. 2020). Furthermore, ischemia causes an increased production of reactive oxygen species (ROS), inflammation, necrosis,

and apoptosis. Ischemia damages CA1 hippocampal neurons, which causes damage to long-term and short-term neurological function and memory (Bachevalier and Meunier 1996; Bendel et al. 2005). The brain is a vulnerable organ to ischemia, as pyramidal neurons in the CA1 region of the hippocampus are sensitive and can be damaged after ischemia (Kiyota et al. 1991). CA1 hippocampal neurons play an important role in cognitive and non-cognitive behaviors, which ischemia can damage, thus causing cognitive problems, such as memory deficit (Hu et al. 2013; Eydipour et al. 2017; Dolatabadi et al. 2019). There are different behavioral tests, such as the Morris water maze, for investigating behavioral changes and memory function after ischemia (D'Hooge and De Deyn 2001; Iihoshi et al. 2004; Gordan et al. 2012). The Morris water test makes it possible to study cognitive deficits following brain injury (Tucker et al. 2018). Our findings in the maze test showed that ischemia damaged CA1 hippocampus neurons and destroyed spatial learning and memory abilities in the ischemia group of rats in comparison with the control group. We investigated memory capability after ischemia and used two strategies to improve memory and learning after ischemia. Studies showed that a short cycle of ischemia-reperfusion could improve short-term and long-term memory and neurological function (Hu et al. 2018; Liu et al. 2019). Remote ischemic preconditioning is a strategy that consist of short cycles of ischemia-reperfusion in a specific limb before the onset of stable ischemia that reduces infarct size through inhibition of oxidation and inflammation (He et al. 2019). Remote ischemic preconditioning could significantly reduce the ischemia reperfusion injury through upregulation and expression of Nrf2 as a transcription factor that regulates the expression of antioxidant proteins (Chen et al. 2018) (Fig. 7). Furthermore, remote ischemic preconditioning was shown to protect against ischemia injury by downregulating the key steps leading to systemic inflammation. This process blocks NFκB, subsequently reducing systemic inflammation (Kim et al. 2014).

Studies have shown that remote ischemic preconditioning can reduce apoptosis by reduction in the expression of caspase-3 and Bax. This reduction showed a decreased incidence of ischemia injury after initial ischemic insult (Wei et al. 2011; Duan et al. 2012; Peng et al. 2012; Park et al. 2016). Kim et al showed that remote ischemic preconditioning was able to increase the survival rate and decrease the tumor necrosis factor (TNFα) level (Kim et al. 2014).

In this study, remote ischemia preconditioning improved CA1 hippocampal neurons and deficits of memory after global ischemia. Atorvastatin is one of the statin group with a neuroprotective effect, which can improve neuronal injury induced by ischemia (Asahi et al. 2005; Hong et al. 2006). Statins cause angiogenesis, neurogenesis, and synaptogenesis after stroke (Chen et al. 2003; Piermartiri et al. 2009). Also, statins can recover memory and learning ability (Baytan et al. 2008; Georgieva-Kotetarova and Kostadinova 2013; Wroolie et al. 2020). Atorvastatin improves the impairment of memory and learning by downregulating the activation of caspase-3 via increasing the phosphorylation of AKt1during ischemia reperfusion (Yang et al. 2015). Our findings showed that global cerebral ischemia created with occlusion of common carotid arteries causes deficits in rat performance, neurodegeneration, and a decreased number of neurons in the hippocampus.
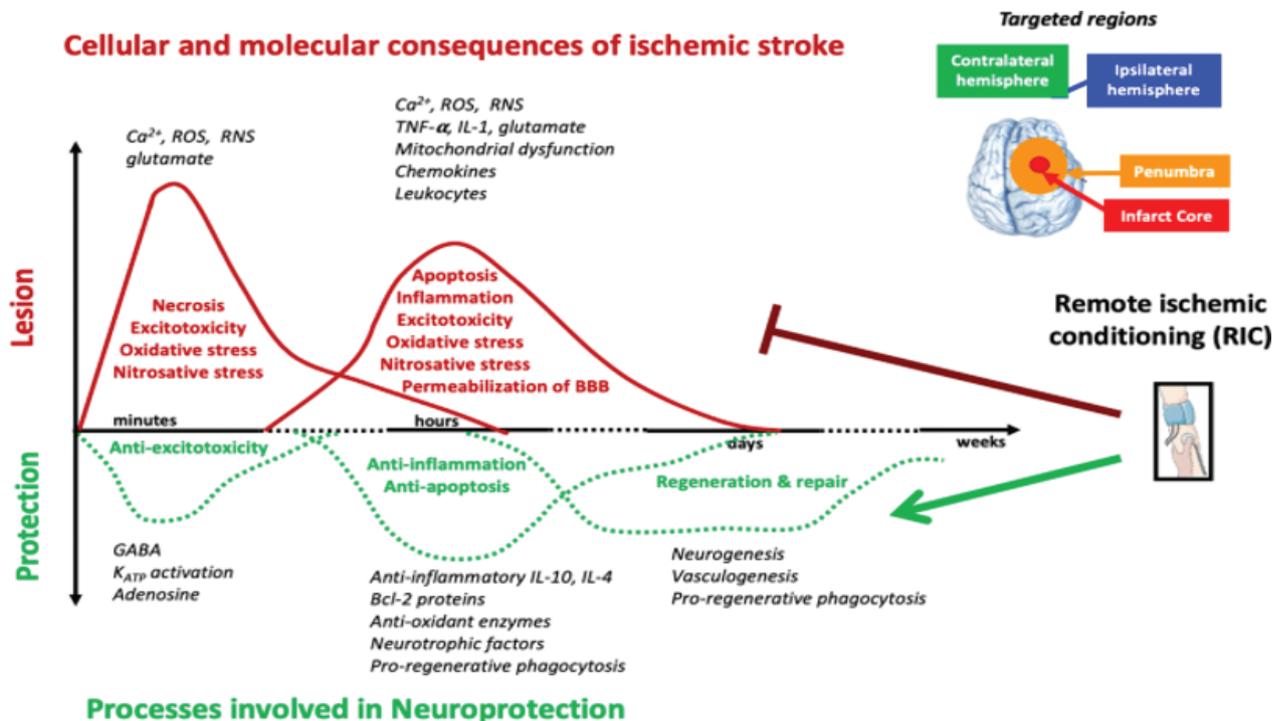

Figure 7. Remote ischemic preconditioning could protect against ischemia reperfusion injury through upregulation and expression of Nrf2.

Our findings are in agreement with the previous studies showing that treatment with atorvastatin could improve neuronal injury of CA1 and improve memory after ischemia (Robin et al. 2014). Our results showed that atorvastatin and remote ischemia preconditioning separately can improve rats' performance, but using remote ischemic preconditioning and atorvastatin together was more effective. In other words, according to the Morris water maze results, the best performance was shown by the group in which remote ischemic preconditioning was used together with atorvastatin. The previous report indicated the effects of remote ischemic preconditioning and atorvastatin on cognitive behavior and memory (Qu et al. 2005; Karki et al. 2009). Our research shows that combined treatment with atorvastatin and remote ischemic preconditioning has a neuroprotective effect on memory and learning of rat, which can be mediated by a modest increase in neurogenesis. In the present study, the animals were subjected to a visible test before conducting the water maze test to ensure the readiness of the animals. All the experimental rats performed the visible tests successfully. In the visible test, all the rats got the similar score. This is consistent with the readiness of all the rats for performing a Morris water maze. In the present study, we investigated working memory (as assessed in STA) and reference memory (probe test) after ischemia using the water maze test (Volpe et al. 1989). Studies have examined spatial reference memory and working memory using the water maze test (Bizon et al. 2009; Kraeuter et al. 2019). We demonstrated that the working memory and reference memory of rats are impaired after ischemia. Reference memory is involved in retaining fixed information, and

reference memory is involved in remembering information for a short time. Memory recovery after stroke can be important at least for two aspects: 1) memory involvement in motor functions, such as verbal performance 2) predicting the severity of stroke (das Nair and Lincoln 2007). Our findings showed that remote ischemic preconditioning and atorvastatin improved rats memory and function in Morris water maze test after ischemia, and that a combination of these strategies is more effective and can significantly recover rats memory.

Conclusion

This study showed that remote ischemic preconditioning and atorvastatin can reduce damage to hippocampal CA1 neurons and improve memory. Remote ischemic pre-conditioning combination therapy with atorvastatin was more effective than their sole use, increasing the number of normal CA1 hippocampal neurons and improving rats function in Morris water maze test.

Conflict of interest

The authors declare that they have no conflict of interests.

Acknowledgment

References

- Asahi M, Huang Z, Thomas S, Yoshimura S, Sumii T, Mori T, Qiu J, Amin-Hanjani S, Huang PL, Liao JK, Lo EH, Moskowitz MA (2005). Protective effects of statins involving both eNOS and tPA in focal cerebral ischemia. Journal of Cerebral Blood Flow & Metabolism 25(6): 722–772. https://doi.org/10.1038/sj.jcbfm.9600070 [PubMed] [PMC]
- Bachevalier J, Meunier M (1996) Cerebral ischemia: are the memory deficits associated with hippocampal cell loss? Hippocampus 6(5): 553–560. https://doi.org/10.1002/(SICI)1098-1063(1996)6:5<553::AID-HIPO8>3.0.CO;2-J [PubMed]
- Baytan SH, Alkanat M, Okuyan M, Ekinci M, Gedikli E, Ozeren M, Akgun A (2008) Simvastatin impairs spatial memory in rats at a specific dose level. The Tohoku Journal of Experimental Medicine 214(4): 341–349. https://doi.org/10.1620/tjem.214.341 [PubMed]
- Bendel O, Bueters T, von Euler M, Ove Ogren S, Sandin J, von Euler G (2005) Reappearance of hippocampal CA1 neurons after ischemia is associated with recovery of learning and memory. Journal of Cerebral Blood Flow & Metabolism 25(12): 1586–1595. https://doi.org/10.1038/sj.jcbfm.9600153 [PubMed]
- Berger C, Xia F, Maurer MH, Schwab S (2008) Neuroprotection by pravastatin in acute ischemic stroke in rats. Brain Research Reviews 58(1): 48–56. https://doi.org/10.1016/j.brainresrev.2007.10.010 [PubMed]
- Bizon JL, LaSarge CL, Montgomery KS, McDermott AN, Setlow B, Griffith WH (2009) Spatial reference and working memory across the lifespan of male Fischer 344 rats. Neurobiology of Aging 30(4): 646–655. https://doi.org/10.1016/j.neurobiolaging.2007.08.004 [PubMed] [PMC]
- Chen G, Thakkar M, Robinson Ch, Doré S (2003) Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. Annals of Neurology 53(6): 743–751. https://doi.org/10.3389/fneur.2018.00040 [PubMed] [PMC]
- Chen G, Thakkar M, Robbinson Ch, Doré S (2018) Limb remote ischemic conditioning: mechanisms, anesthetics, and the potential for expanding therapeutic options. Frontiers in Neurology 9: 40.
- D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain Research Reviews 36(1): 60–90. https://doi.org/10.1016/S0165-0173(01)00067-4 [PubMed]
- Ding ZM, Wu B, Zhang WQ, Lu XJ, Lin YC, Geng YJ, Miao YF (2012) Neuroprotective effects of ischemic preconditioning and postconditioning on global brain ischemia in rats through the same effect on inhibition of apoptosis. International Journal of Molecular Sciences 13(5): 6089–6101. https://doi.org/10.3390/ijms13056089 [PubMed] [PMC]

- Duan X, Ji B, Wang X, Liu J, Zheng Z, Long C, Tang Y, Hu S (2012) Expression of microRNA-1 and microRNA-21 in different protocols of ischemic conditioning in an isolated rat heart model. Cardiology 122(1): 36–43. https://doi.org/10.1159/00033814 [PubMed]

- Eydipour Z, Vaezi G, Nasehi M, Haeri-Rouhani SA, Zarrindast MR (2017) Different role of CA1 5HT3 serotonin receptors on memory acquisition deficit induced by total (TSD) and REM sleep deprivation (RSD). Archives of Iranian Medicine 20(9): 581–588. https://doi.org/10.15171/icnj.2018.04

- Farovik A, Dupont LM, Eichenbaum H (2010) Distinct roles for dorsal CA3 and CA1 in memory for sequential nonspatial events. Learning & Memory 17(1): 12–17. https://doi.org/10.1101/lm.1616209 [PubMed] [PMC]

- Georgieva-Kotetarova MT, Kostadinova II (2013) Effect of atorvastatin and rosuvastatin on learning and memory in rats with diazepam-induced amnesia. Folia Medica 55(2): 58. https://doi.org/10.2478/folmed-2013-0018 [PubMed]

- Godinho J, de Oliveira RMW, de Sa-Nakanishi AB, Bacarin CC, Huzita CH, Longhini R, Mello JCP, Nakamura CV, Previdelli IS, Dal Molin Ribeiro MH, Milani H (2018) Ethyl-acetate fraction of Trichilia catigua restores long-term retrograde memory and reduces oxidative stress and inflammation after global cerebral ischemia in rats. Behavioural Brain Research 337: 173–182. https://doi.org/10.1016/j.bbr.2017.08.050 [PubMed]

- Gordan ML, Jungwirth B, Ohl F, Kellermann K, Kochs EF, Blobner M (2012) Evaluation of neurobehavioral deficits following different severities of cerebral ischemia in rats: a comparison between the modified hole board test and the Morris water maze test. Behavioural Brain Research 235(1): 7–20. https://doi.org/10.1016/j.bbr.2012.07.027 [PubMed]

- He JT, Li H, Yang L, Cheng KL (2019) Involvement of endothelin-1, H 2 S and Nrf2 in beneficial effects of remote ischemic preconditioning in global cerebral ischemia-induced vascular dementia in mice. Cellular and Molecular Neurobiology 39(5): 671–686. https://doi.org/10.1007/s10571-019-00670-y [PubMed]

- Hong H, Zeng JS, Kreulen DL, Kaufman DI, Chen AF (2006) Atorvastatin protects against cerebral infarction via inhibition of NADPH oxidase-derived superoxide in ischemic stroke. American Journal of Physiology-Heart and Circulatory Physiology 291(5): H2210–H2215. https://doi.org/10.1152/ajpheart.01270.2005 [PubMed]

- Hu X, Lu Y, Zhang Y, Li Y, Jiang L (2013) Remote ischemic preconditioning improves spatial learning and memory ability after focal cerebral ischemia-reperfusion in rats. Perfusion 28(6): 546–551. https://doi.org/10.1177/0267659113487766 [PubMed]

- Hu X, Lv T, Yang SF, Zhang XH, Miao YF (2018) Limb remote ischemic post-conditioning reduces injury and improves long-term behavioral recovery in rats following subarachnoid hemorrhage: Possible involvement of the autophagic process. Molecular Medicine Reports 17(1): 21–30. https://doi.org/10.3892/mmr.2017.7858 [PubMed] [PMC]

- Iihoshi S, Honmou O, Houkin K, Hashi K, Kocsis JD (2004) A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. Brain Research 1007(1–2): 1–9. https://doi.org/10.1016/j.brainres.2003.09.084 [PubMed]

- Kamali Dolatabadi L, Emamghoreishi M, Namavar MR, Badeli Sarkala H (2019) Curcumin effects on memory impairment and restoration of irregular neuronal distribution in the hippocampal

- CA1 region after global cerebral ischemia in male rats. Basic and Clinical Neuroscience 10(5): 527–539. https://doi.org/10.32598/bcn.9.10.365 [PubMed] [PMC]

- Karki K, Knight RA, Han Y, Yang D, Zhang J, Ledbetter KA, Chopp M, Seyfried DM (2009) Simvastatin and atorvastatin improve neurological outcome after experimental intracerebral hemorrhage. Stroke 40(10): 3384–3389. https://doi.org/10.1161/STROKEAHA.108.544395 [PubMed] [PMC]

- Kesner RP, Hunsaker MR, Ziegler W (2010) The role of the dorsal CA1 and ventral CA1 in memory for the temporal order of a sequence of odors. Neurobiology of Learning and Memory 93(1): 111–116. https://doi.org/10.1016/j.nlm.2009.08.010 [PubMed]

- Kim YH, Yoon DW, Kim JH, Lee JH, Lim CH (2014) Effect of remote ischemic post-conditioning on systemic inflammatory response and survival rate in lipopolysaccharide-induced systemic inflammation model. Journal of Inflammation 11(1): 1–9. https://doi.org/10.1186/1476-9255-11-1 [PubMed] [PMC]

- Kiyota Y, Miyamoto M, Nagaoka A (1991) Relationship between brain damage and memory impairment in rats exposed to transient forebrain ischemia. Brain Research 538(2): 295–302. https://doi.org/10.1016/0006-8993(91)90443-y [PubMed]

- Kraeuter AK, Guest PC, Sarnyai Z (2019) The Y-maze for assessment of spatial working and reference memory in mice. Methods in Molecular Biology 1916: 105–111. https://doi.org/10.1007/978-1-4939-8994-2_10 [PubMed]

- Lee TK, Kang IJ, Kim B, Sim HJ, Kim DW, Ahn JH, Lee JC, Ryoo S, Shin MC, Cho JH, Kim YM, Park JH, Choi SY, Won MH (2020) Experimental pretreatment with chlorogenic acid prevents transient ischemia-induced cognitive decline and neuronal damage in the hippo campus through anti-oxidative and anti-inflammatory effects. Molecules 25(16): e3578. https://doi.org/10.3390/molecules25163578 [PubMed] [PMC]

- León-Moreno LC, Castañeda-Arellano R, Rivas-Carrillo JD, Dueñas-Jiménez SH (2020) Challenges and improvements of developing an ischemia mouse model through bilateral common carotid artery occlusion. Journal of Stroke and Cerebrovascular Diseases 29(5): e104773. https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.104773 [PubMed]

- Liu Y, Li AQ, Ma W, Gao YB, Deng LQ, Zhang C, Meng JH (2019) Limb remote ischemic preconditioning reduces repeated ketamine exposure-induced adverse effects in the developing brain of rats. Journal of Molecular Neuroscience 68(1): 58–65. https://doi.org/10.1007/s12031-019-01282-3 [PubMed]

- Lugtmeijer S, Lammers NA, de Haan EHF, de Leeuw FE, Kessels RP (2021) Post-stroke working memory dysfunction: A meta-analysis and systematic review. Neuropsychology Review 31(1): 202–219. https://doi.org/10.1007/s11065-020-09462-4 [PMC]

- Mounier NM, Wahdan SA, Gad AM, Azab SS (2021) Role of inflammatory, oxidative, and ER stress signaling in the neuroprotective effect of atorvastatin against doxorubicin-induced cognitive impairment in rats. Naunyn-Schmiedeberg's Archives of Pharmacology 394(7): 1537–1551. https://doi.org/10.1007/s00210-021-02081-7 [PubMed]

- Nair RD, Lincoln NB (2007) Cognitive rehabilitation for memory deficits following stroke. Cochrane Database of Systematic Reviews 3: CD002293. https://doi.org/10.1002/14651858.CD002293.pub2 [PubMed]

- Ólafsdóttir HF, Bush D, Barry C (2018) The role of hippocampal replay in memory and planning. Current Biology 28(1): R37–R50. https://doi.org/10.1016/j.cub.2017.10.073 [PubMed] [PMC]
- Park MS, Joo SH, Kim BS, Lee JW, Kim YI, Hong MK, Ahn HJ (2016) Remote preconditioning on rat hepatic ischemia-reperfusion injury downregulated Bax and cleaved caspase-3 expression. Transplantation Proceedings 48(4): 1247–1250. https://doi.org/10.1016/j.transproceed.2015.12.125 [PubMed]
- Peng B, Guo QL, He ZJ, Ye Z, Yuan YJ, Wang N, Zhou J (2012) Remote ischemic postconditioning protects the brain from global cerebral ischemia/reperfusion injury by up-regulating endothelial nitric oxide synthase through the PI3K/Akt pathway. Brain Research 1445: 92–102. https://doi.org/10.1016/j.brainres.2012.01.033 [PubMed]
- Pérez-Pinzón MA (2004) Neuroprotective effects of ischemic preconditioning in brain mitochondria following cerebral ischemia. Journal of Bioenergetics and Biomembranes 36(4): 323–327. https://doi.org/10.1023/B:JOBB.0000041762.47544.ff [PubMed]
- Piermartiri TC, Vandresen-Filho S, de Araújo Herculano B, Martins WC, Dal'agnolo D, Stroeh E, Carqueja CL, Boeck CR, Tasca CI (2009) Atorvastatin prevents hippocampal cell death due to quinolinic acid-induced seizures in mice by increasing Akt phosphorylation and glutamate uptake. Neurotoxicity Research 16(2): 106–115. https://doi.org/10.1007/s12640-009-9057-6 [PubMed]
- Qu C, Lu D, Goussev A, Schallert T, Mahmood A, Chopp M (2005) Effect of atorvastatin on spatial memory, neuronal survival, and vascular density in female rats after traumatic brain injury. Journal of Neurosurgery 103(4): 695–701. https://doi.org/10.3171/jns.2005.103.4.0695 [PubMed]
- Ren C, Liu Y, Stone C, Li N, Li S, Li H, Cheng Z, Hu J, Li W, Jin K, Ji X, Ding Y (2021) Limb remote ischemic conditioning ameliorates cognitive impairment in rats with chronic cerebral hypoperfusion by regulating glucose transport. Aging and Disease 12(5): 1197–1210. https://doi.org/10.14336/AD.2020.1125 [PubMed] [PMC]
- Robin NC, Agoston Z, Biechele TL, James RG, Berndt JD, Moon RT (2014) Simvastatin promotes adult hippocampal neurogenesis by enhancing Wnt/β-catenin signaling. Stem Cell Reports 2(1): 9–17. https://doi.org/10.1016/j.stemcr.2013.11.002 [PubMed] [PMC]
- Tucker LB, Velosky AG, McCabe JT (2018) Applications of the Morris water maze in translational traumatic brain injury research. Neuroscience & Biobehavioral Reviews 88: 187–200. https://doi.org/10.1016/j.neubiorev.2018.03.010 [PubMed]
- Veighey K, Macallister RJ (2012) Clinical applications of remote ischemic preconditioning. Cardiology Research and Practice 2012: 620681. https://doi.org/10.1155/2012/620681 [PubMed] [PMC]
- Volpe BT, Davis HP, Colombo PJ (1989) Preoperative training modifies radial maze performance in rats with ischemic hippocampal injury. Stroke 20(12): 1700–1706. https://doi.org/10.1161/01.str.20.12.1700 [PubMed]
- Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols 1(2): 848–858. https://doi.org/10.1038/nprot.2006.116 [PubMed] [PMC]
- Wang M, Qi DS, Zhou C, Han D, Li PP, Zhang F, Zhou XY, Han M, Di JH, Ye JS, Yu HM, Song YJ, Zhang GY (2016) Ischemic preconditioning protects the brain against injury via inhibiting CaMKII–nNOS signaling pathway. Brain Research 1634: 140–149. https://doi.org/10.1016/j.brainres.2016.01.008 [PubMed]
- Wei M, Xin P, Li S, Tao J, Li Y, Li J, Liu M, Li J, Zhu W, Redington AN (2011) Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction. Circulation Research 108(10): 1220–1225. https://doi.org/10.1161/CIRCRESAHA.110.236190 [PubMed]
- Wroolie T, Roat-Shumway S, Watson K, Reiman E, Rasgon N (2020) Effects of LDL cholesterol and statin use on verbal learning and memory in older adults at genetic risk for alzheimer's disease. Journal of Alzheimer's Disease 75(3): 903–910. https://doi.org/10.3233/JAD-191090 [PubMed]
- Yang J, Pan Y, Li X, Wang X (2015) Atorvastatin attenuates cognitive deficits through Akt1/caspase-3 signaling pathway in ischemic stroke. Brain Research 1629: 231–239. https://doi.org/10.1016/j.brainres.2015.10.032 [PubMed]

Author Contribution

- **Azim Hedayatpour**, Ph.D. in Anatomy, Associate Professor, Department of Anatomy, e-mail: hedayatpour@tums.ac.ir, **ORCID ID** https://orcid.org/0000-0001-8509-9764. This author performed the experiments and analyzed the results.

- **Maryam Shiasi**, Ph.D. in Anatomy, Department of Anatomy, e-mail: s.shiasi@gmail.com, **ORCID ID** https://orcid.org/0000-0003-1689-9623. This author performed the water maze test and analyzed the data.

- **Peyman Modarresi**, Dr. of Veterinary Medicine, School of Medicine, e-mail: peyman.modarresi@yahoo.com, **ORCID ID** https://orcid.org/0000-0001-5875-4719. This author designed the animal surgery procedure, performed the surgery and analyzed data.

- **Alieh Bashghareh**, PhD student, Department of Anatomy, e-mail: alieh.bashghare@yahoo.com, **ORCID ID** https://orcid.org/0000-0002-5866-7268. This author designed the idea of the study and wrote the article.