4-Methyllumbiferone (4-MU) exerts a neuroprotective effect against cerebral ischemia/reperfusion injury by ameliorating learning and memory impairments

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Declarations

Ethics approval and consent to participate

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Consent for publication

Not Applicable

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Availability of data and material

The findings of this study are derived from data that are available upon request from the corresponding authors.

Competing interests

The authors declare no conflicts of interest.

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Abstract

Background: Stroke is the sixth leading cause of death and lifelong disability for millions of people in the United States. Cerebral ischemia lead to in oxidative stress, excitotoxicity, inflammation, apoptosis and also, impairments in memory and learning occur in majority of subjects with ischemic stroke. Lack of definitive treatment has sparked extensive research into novel therapeutic strategies, including the use of 4-methylumbelliferone (4-MU), a coumarin derivative with potential neuroprotective properties. The present study examines impact of 4-MU on reducing cerebral ischemia-reperfusion (I/R) injury and learning and memory impairments in male Wistar rats.

Method: The animal was exposed to middle cerebral artery occlusion (MCAO) and were cured with one dose of 4-MU (at dosage of 25 mg/kg) dissolved in DMSO 0.9%. Automated shuttle box test was employed to evaluate learning and memory impairments. Western blot assay, TTC staining and Nissl staining was used to measure protein expression, infarct volume, and cell death, respectively.

Results: Results showed treatment with 4-MU reduced infarct volume and improved learning and memory impairments by down-regulation of HAS1 and HAS2. 4-MU has been shown to modulate the release of pro-inflammatory cytokines include TNF-α and IL-1β, as well as anti-inflammatory markers like IL-10 and also reduced oxidative stress markers in the brain.

Conclusion: Generally, neuroprotective effects of 4-MU against cerebral I/R injury can be attributed to down-regulation of HAS1 and 2.

Keywords: Ischemic stroke; MCAO; Learning and memory impairments; 4-methylumbelliferone; Neural death; Hyaluronan synthases

Abbreviations

4-MU: 4-methylumbelliferone

MCAO: Middle Cerebral Artery Occlusion

HAS: Hyaluronan Synthases

I/R: Ischemia-reperfusion

1. Introduction

One of the main outcomes following ischemic stroke is impairments in memory and learning that strongly influences patients' life quality (1). Previous investigations have shown that cerebral

ischemia for a short time (less than 10 min) contribute to initiation of cell death in the hippocampus and causing deficits in learning and memory functions (2).

A large number of individuals who survive from the acute phase of ischemic stroke encounter some adverse effects relevant to deficits in memory and learning since available treatments are no completely effective (3). According to the clinical studies, immediate or delayed dysfunctions in memory and learning occur in 25–30% of post-stroke survivors which in turn heavily influences rehabilitation programs for performing performances of daily living (4, 5). Hyaluronan (HA) has been found to be a key ingredient of the brain extracellular matrix and a crucial player in numerous processes like angiogenesis, cellular proliferation, differentiation, and migration. Increased synthesis of total HA and low molecular mass 3–10 disaccharides of HA (o-HA) in the serum of patients with ischemic stroke (1, 3, 7 and 14 days) and post-mortem tissues have been reported in a prior investigation, mainly attributed to overexpression of HA synthases (HAS1 and 2) and hyaluronidases in inflammatory cells in peri-infarcted areas of the cerebral tissue (6). Elevated expression of hyaluronidase 1 and HAS2 in astrocytes following ischemic stroke aggravate infarct volume and suppressing hyaluronidase activity early after stroke can contribute to functional recovery (7).

It has been found that 4-methylumbelliferone (4-MU), an inhibitor of hyaluronic acid synthesis, has protective impact against ischemia/reperfusion injury and other disorders by inhibition of oxidative stress and inflammation (8, 9, 10, 11, 12). Protective impact of 4-MU is associated with reducing macrophage invasion (13).

The accumulating evidence suggests that 4-MU may be a valuable adjunctive therapy for treatment of ischemic stroke and other serious diseases (14, 15, 16). Its multifaceted mechanism

of action, encompassing anti-inflammatory, antioxidant, and anti-apoptotic properties, may provide a therapeutic advantage over single-target agents (17). Ultimately, the development of 4-MU as a therapeutic agent for ischemic stroke may contribute to improved patient outcomes and a reduction in the socioeconomic burden of this devastating disease. Here we tested whether 4-MU is capable of reducing cerebral I/R injury by activities of HAS1 and 2 in a rat model.

2. Materials and Methods

2.1. Animals and Experimental Design

Wistar rats (male, 275 ± 15 g) were purchased from the Animal Center of Iran University of Medical Sciences (IUMS). Controlled conditions including 12h light-dark schedule at a temperature of 22 ± 2°C, without restrictions to standard food and water ad libitum were selected for keeping he animals. All protocols and procedures relevant to animals were authorized by the Ethics Committee of IUMS and were performed under the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

80 rats were randomly assigned into four groups (n=20, per groups): Sham, I/R, vehicle, and 4-MU (25 mg/kg). In sham group, the animals only were subjected to surgical shock. In I/R group, the animals were subjected to MCAO for 60 min and then reperfusion was allowed for 24 h. In vehicle group, the animals were subjected to MCAO for 60 min and at the onset of reperfusion, solvent (DMSO 0.9%) was administrated intraperitoneally. In 4-MU group, the animals were subjected to MCAO for 60 min and at the onset of reperfusion, 4-MU (at dosage of 25 mg/kg) dissolved in DMSO 0.9% was intraperitoneally administrated.

2.2. Ischemic Stroke Model (MCAO)

The MCAO/R model was used to induce model as describe by Longa et al (18). The animals were anesthetized using a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally. After isolation of the right common artery, external carotid artery (ECA), and internal carotid artery (ICA), the distal end of the ECA was cut and a 4–0 silicon rubber-coated monofilament containing a tapered end was inserted into the right ICA and forwarded approximately 18 ± 2 mm to feel slight resistance as an indicator of blocking MCA. To permit reperfusion, silicon rubber-coated monofilament was taken out after 60 min. similar surgical procedures were performed for sham animals except inserting silicon rubber-coated monofilament.

2.3. Automated Shuttle Box Test

A shuttle box including a two-part plexiglass box with a bright section and a dark section, and the equal dimensions of the two sections (20×20×40 cm) was employed to assess learning and memory function. Instrument includes stainless steel bars on the floor of both section with a 1 cm distance, a 100-W light bulb 40 cm above the device's bright side, and a guillotine door between 2 sections. The experiment was carried out in 3 steps: 1) adaption: each rat was habituated with apparatus for ≥5 min in two consecutive days prior the start of the test. 2) Acquisition: in the third day, each rat was located in bright section, and the chamber is kept dark for 2 min. both dark and bright sections are separated by a guillotine door. at the end of the period and after turning on the chamber's light and opening the guillotine door, the time it takes for the entering animal from the light to the dark chamber is recorded by the chronometer and describes as "Initial latency". Then, after closing the door, a single electric shock (1 mA for 1 second) is exerted to the rat. One min after the end of the experiment, the rat is transferred to the cage. At this step, animals with a delay more than 60 seconds are excluded from the experiment.

The retention and recall step were done 24 h after the second step on the fourth day. This step is like prior step, and the difference is that the rat was not given any shock when it enters the dark section. STL (step-through latency) is recorded at this point. STL is an indicator of the time the animal stays in the light section prior going the dark section. Cut-off time was 480s if the animal did move the dark section.

2.4. Morris water maze (MWM) test

Spatial learning and memory performance was investigated by MWM. The maze used in this study includes a black circular pool (diameter = 150 cm, height = 60 cm) filled with 20 ± 1°C water to a height of 25 cm. four quadrants of the black circular pool are arbitrarily described as southeast, and southwest northeast, and northwest. In the target quadrant (southeast), a hidden circular platform (diameter = 10 cm and 1.5 cm below the water surface) was put. During all days of the test, visual cues were put around the maze and recording the swimming paths of rats during trials was performed using a camera fixed above the center of the maze. Before triggering each daily test, rats were placed in a dark test room for 30 min. Four trials each day for three consecutive days was performed for each animal. Each animal was subjected to a 20 s habituation time on the platform just on the first day. The rats were put in the water facing the maze wall and allowed to swim for 90 s until they discovered the platform at initiation of all trials. The rats with lack of ability for exploring the platform were manually put to it. After reaching the platform, they were permitted to remain for 20 s on the platform and then come back to their cage. A random selection for triggering positions was done each day. The probe (retention test) and visible test were performed on day 4. To carry out the probe test, the escape platform was removed from the maze. Afterward, the rats were located in the water from the opposite point to the target quadrant and allowed to swim for 60 s. sensorimotor

abilities of animals were measured using a visible platform test after the probe trial. Processing and analysis of the recorded behaviors of each rat was done using a computerized system (Noldus EthoVision, 11.1 version).

2.5. TTC staining

The brain infarct volume was measured using TTC staining. After sacrificing of animals at the end of experiment, brain tissues were separated and cut into 2 mm coronal segments. To perform staining, the brain sections were immersed in 2% TTC for 15 min at 37 □ and fixed with 4% paraformaldehyde to increase contrast before taking photographs. Calculation of the percentage of infarct volume was done based on following formula:

Brain infarct volume (%) = [left hemisphere volume − (right hemisphere volume − infarct volume)] / left hemisphere volume × 100 %.

2.6. Western blotting

Rat brain tissue proteins were extracted from infarcted regions using the RIPA lysis buffer. a BCA protein assay kit was employed to measure the protein concentrations. After separation of A 50μg protein of each sample based on molecular weight by electrophoresis, they were transferred into PVDF membranes. After placing the membranes into primary antibodies against HAS1 and 2 overnight at 4 °C, they were incubated with corresponding HRP-conjugated secondary antibodies for 2 hours at 37 □. The color development of the PVDF membranes was done using the chemiluminescent HRP Substrate (Millipore). Quantification of the values of protein was performed using Image-J software. β-actin was employed as the loading control.

2.7. Histopathological examinations

Histopathological alterations of cerebral tissues were determined using Nissl staining. The brain tissues were fixed with 4% paraformaldehyde, embedded in paraffin sections, and cut in 5 µm slides. Then the brain tissues were Nissl stained, and sealed. An optical microscope (Motic China Group Co., Ltd., BA210Digital, Xiamen, China) was used o investigate the pathological alterations in the CA1 region of the hippocampus.

2.8. Statistical analysis

The findings were defined as mean ±□Standard error of the mean (SEM). Analysis of all data was done by using one-way ANOVA followed by Bonferroni's test as a post hoc analytical test (GraphPad Prism 9.0 software). P-value less than 0.05 shows a statistically significant difference.

3. Results

3.1. Infarct Volume

The obvious cerebral infarction was evident in I/R cohort compared to sham, indicating the successful establishment of stroke model. As expected, a significant reduction in infarct size was observed in MCAO rats treated with 4-MU (25 mg/kg) (Figure 1).

Sham I/R Vehicle 4-MU

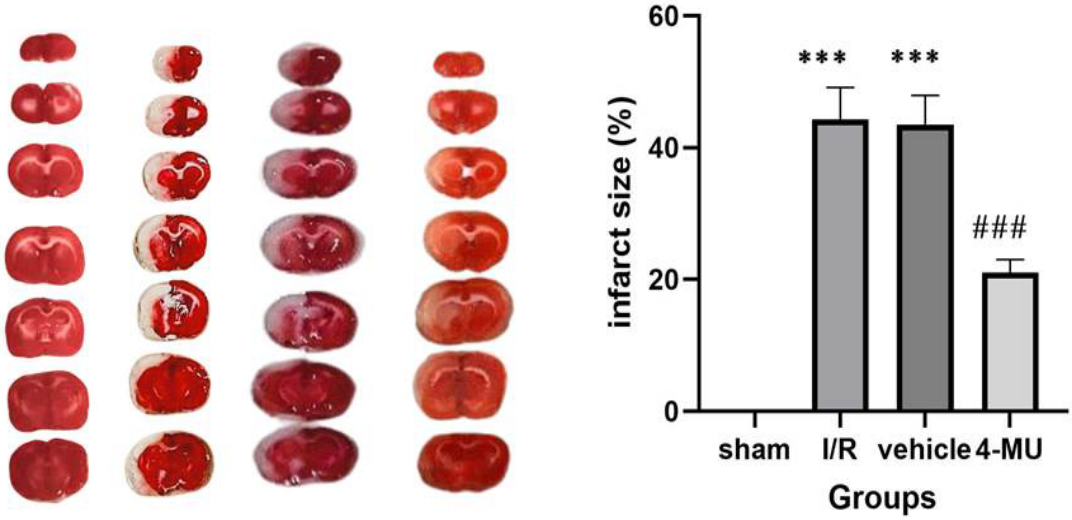

Figure 1. Representative images of TTC staining in different groups. Red color shows healthy regions while white color demonstrates infarcted regions (n=5 per group). ***p□<□0.001 vs. sham; ### p□<□0.001 vs. I/R and vehicle groups). Data were expressed as mean ±□SEM.

3.2. Learning and memory

Remarkable reductions in STL were observed in animals exposed to MCAO compared to sham. 4-MU (25 mg/kg) could contribute to restoration of STL compared to I/R and vehicle groups. Moreover, MCAO rats exhibited significantly higher time in dark chamber than sham. Treatment with only DMSO 0.9% did no change time in dark chamber whereas treatment with 4-MU (25 mg/kg) markedly decreased time in dark chamber than I/R and vehicle cohorts (Figure 2 A-B).

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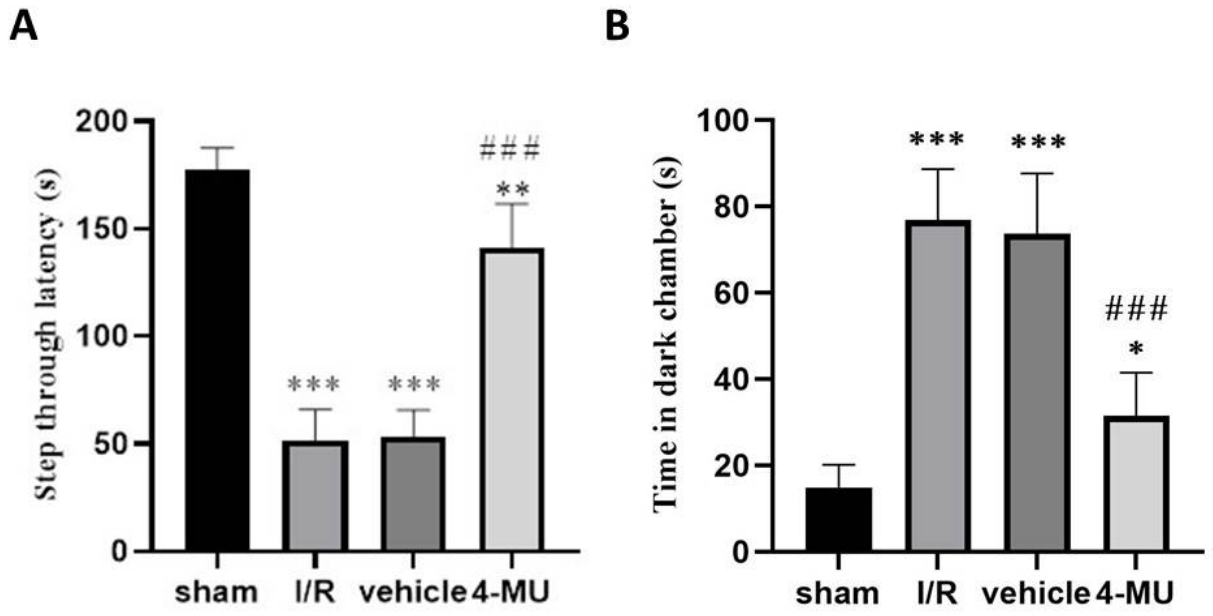

Figure 2. Impact of 4-MU on ischemic stroke-induced learning and memory impairments. **A)** STL, **B)** time in dark chamber (n=7). ***p□<□0.001, **p□<□0.01, and *p□<□0.05 vs. sham; ### p□<□0.001 vs. I/R and vehicle groups). Data were expressed as mean ±□SEM.

3.3 Behavioral parameters in the MWM test

Reduced levels of the time spent in the goal quarter and elevated amount of latency time were observed in I/R and vehicle cohorts versus sham (Figure 3 A-B). Treatment of I/R rats with 4-MU (25 mg/kg) markedly improved the time spent in the goal quarter and reduced latency time versus I/R and vehicle cohorts. There were no remarkable differences between the various cohorts for speed index (Figure 3 C).

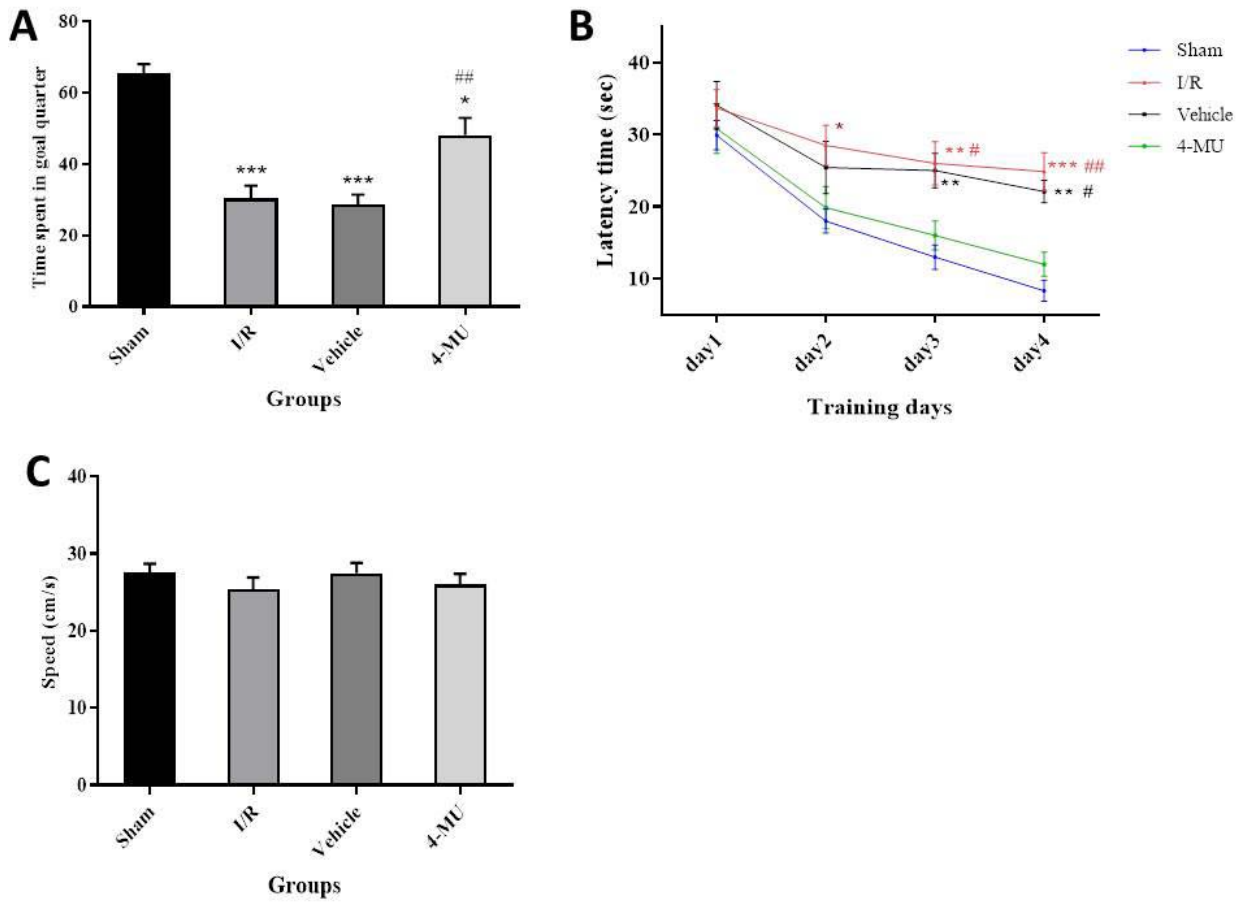

Figure 3. Impact of 4-MU on behavioral parameters in the Morris water maze (MWM) test (n□=□7 per group). **A)** The time spent in the goal quarter, **B)** Latency time, and **C)** Speed index. *□**□p□<□0.001, *□*□p□<□0.01, *□p□<□0.05 vs. sham; ## p□<□0.01 and #p□<□0.05 vs. I/R and vehicle cohorts). Data were expressed as mean ±□SEM.

3.4. Expression of HAS1 and 2

HAS1 and 2 as targets of 4-MU were detected to be up-regulated following MCAO than sham. The animals treated with 4-MU (25 mg/kg) demonstrated reduced expression levels of both HAS1 and 2 in comparison with I/R and vehicle cohorts (Figure 4 A-B).

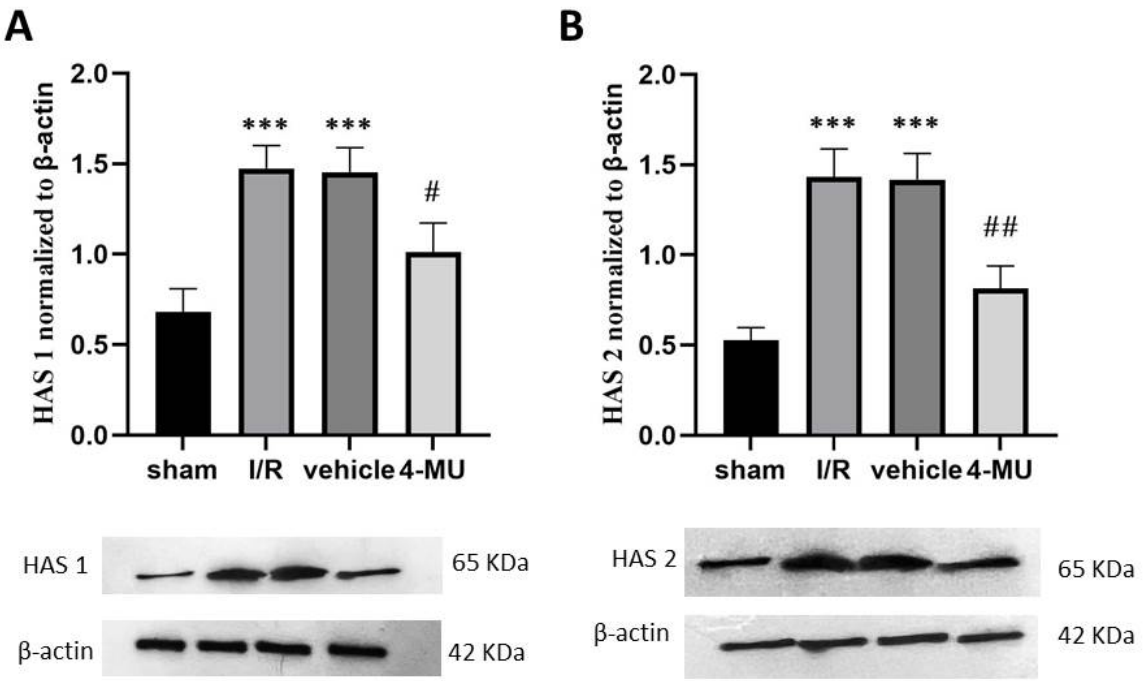

Figure 4. Western blot for detection of the expression of A) HAS1, B) HAS 2 in brain tissues (n = 5). ***p□<□0.001 vs. sham; ## p□<□0.01 and # p□<□0.05 vs. I/R and vehicle groups. Data were expressed as mean ±□SEM.

3.5. Neuronal density

Nissl staining of the CA1 region of the hippocampus indicated the intact and tightly arranged neuronal cells with clear cytoplasm and nucleoli with a monotonous staining. Scattered arrangement and nuclear deformation were observed in I/R and vehicle cohorts than sham. Treatment with 4-MU (25 mg/kg) markedly prevented damage to cells (Figure 5).

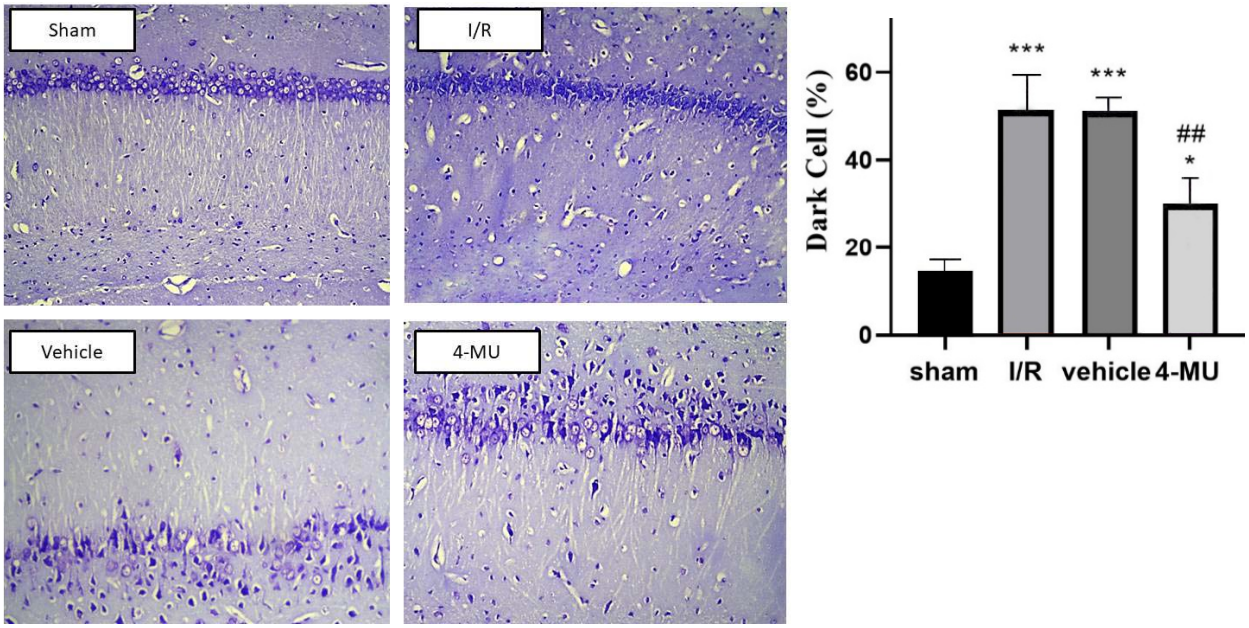

Figure 5. Representative images of Nissl staining in CA1 region of hippocampus (Scale bar, 50 μm) (n=5). ***p□<□0.001 vs. sham; ## p□<□0.01 and # p□<□0.05 vs. I/R and vehicle groups. Data were expressed as mean ±□SEM

4. Discussion

In addition to physical disability, cerebral I/R injury causes learning and memory impairments which heavily influence the functional recovery post-stroke (6, 19). In the present study, our findings indicated that treatment with 4-MU (25 mg/kg) attenuated infarct volume and reductions in STL and elevated time in dark chamber in MCAO that shows learning and memory improvement. Consistent with our results, Dubisova et al. reported that oral administration of 4-MU attenuated perineuronal nets and improved recognition memory in mice (20).

Moreover, we found that beneficial effects of 4-MU in coping with I/R injury is associated with inhibition of both HAS1 and HAS2. Emerging evidence demonstrate that 4-MU is capable of reducing damage following I/R by suppressing oxidative stress and down-regulation of

TLR4/NF-κB/NLRP3 (21). Hyaluronic acid is a main ingredient of the brain extracellular matrix and a modulator of cell differentiation, migration, and angiogenesis. Prior investigations have found that hyaluronan accumulation in stroke-influenced regions is linked to overexpression of hyaluronidase-1 and 2 one□hour-21 days after stroke (22). A clinical study by Al'Qteishat et al. showed that the expression and concentrations of the enzymes responsible for HA synthesis and degradation markedly increased in post-mortem tissue and in the serum of patients 1, 3, 7 and 14 days following stroke (6). During homeostatic circumstance, low HA-binding capacity has been reported for most immune cells. In inflammation conditions, elevated HA binding of activated immune cells occurs by CD44 (20, 21). Increased expression of HAS isoenzymes (HAS1–3) during inflammation contributes to a preponderance of low-molecular-weight and hence activation of macrophages through TLR which in turn result in elevation of inflammation at site of injury (22). These previous investigations are compatible with data herein that indicated tissue damage by MCAO resulted in up-regulation of HAS1 and HAS2 and subsequently obvious infarct volume and cell death in the CA1 region of the hippocampus. Such abnormalities were reversed by treatment with 4-MU (25 mg/kg), suggesting protective effects of 4-MU against cerebral I/R injury by inhibiting HAS1 and HAS2.

5. Conclusion

Ischemic stroke, a leading cause of morbidity and mortality worldwide, is characterized by the obstruction of blood flow to the brain, resulting in neuronal death and tissue damage. HAS enzymes been responsible for the synthesis of HA, a glycosaminoglycan that plays a crucial role in the regulation of inflammation, cell migration, and tissue repair. 4-MU is a naturally occurring compound that has been shown to inhibit HAS activity, thereby reducing HA production. The

purpose of this study is to investigate the current understanding of the role of HAS in ischemic stroke and to discuss the potential therapeutic effects of 4-MU on ischemic stroke through the inhibition of HAS. The neuroprotective effects of 4-MU in ischemic stroke are thought to be mediated through the inhibition of HAS and the subsequent reduction in HA production. Also, 4-MU has been shown to reduce infarct size, improve neurological function, and enhance cognitive recovery in animal models of MCAO. In this study, we found here that 4-MU may be a good candidate to reduce cerebral I/R injury and improve learning and memory impairments in a rat model of ischemic stroke. It seems likely that neuroprotective impact of 4-MU in coping with cerebral I/R injury is related to suppressing activities of HAS1 and 2.

Declarations:

Ethics approval and consent to participate

All protocols were approved by Ethical Committee of Iran University of Medical Sciences, Tehran, Iran.

Consent for publication

All authors agree to publish article in the present form

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

N.A participated in the design and interpretation of the study, data analysis, and the review of the manuscript. SH.T, H.M-J and F.R conducted the experiment and collected the tissue and was responsible for the data analysis. SH.T performed histopathological analyses. SH.T and F.R wrote the manuscript, and all authors reviewed, read, and approved the article.

References

1. Jokinen H, Melkas S, Ylikoski R, Pohjasvaara T, Kaste M, Erkinjuntti T, et al. Post-stroke cognitive impairment is common even after successful clinical recovery. European journal of neurology. 2015;22(9):1288-94.
2. Bacigaluppi M, Comi G, Hermann DM. Animal models of ischemic stroke. Part two: modeling cerebral ischemia. The open neurology journal. 2010;4:34.
3. Van Der Flier WM, Skoog I, Schneider JA, Pantoni L, Mok V, Chen CL, et al. Vascular cognitive impairment. Nature reviews Disease primers. 2018;4(1):1-16.
4. Pantoni L. Have stroke neurologists entered the arena of stroke-related cognitive dysfunctions? Not yet, but they should! : Am Heart Assoc; 2017. p. 1441-2.
5. Kalaria RN, Akinyemi R, Ihara M. Stroke injury, cognitive impairment and vascular dementia. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2016;1862(5):915-25.
6. Al'Qteishat A, Gaffney J, Krupinski J, Rubio F, West D, Kumar S, et al. Changes in hyaluronan production and metabolism following ischaemic stroke in man. Brain. 2006;129(8):2158-76.
7. Katarzyna Greda A, Nowicka D. Hyaluronidase inhibition accelerates functional recovery from stroke in the mouse brain. Journal of Neurochemistry. 2021;157(3):781-801.
8. . !!! INVALID CITATION !!! (Vigetti, Rizzi et al. 2009, Fang, Wang et al. 2014, McKallip, Ban et al. 2015, Chistyakov, Nikolskaya et al. 2020, Moradi, Aslani et al. 2024).
9. Hackert K, Homann S, Mir S, Beran A, Gorreßen S, Funk F, et al. 4-methylumbelliferone attenuates macrophage invasion and myocardial remodeling in pressure-overloaded mouse hearts. Hypertension. 2021;77(6):1918-27.
10. Jahangiri HM, Moradi A, Nazarinia D, Aboutaleb N. 4-methylumbilliferon (4-MU) as a Potential Treatment Against Cerebral ischemia and Reperfusion Injury in Rats; An Experimental Study. Archives of Academic Emergency Medicine. 2024;13(1):e8.
11. Pibuel MA, Díaz M, Molinari Y, Poodts D, Silvestroff L, Lompardía SL, et al. 4-Methylumbelliferone as a potent and selective antitumor drug on a glioblastoma model. Glycobiology. 2021;31(1):29-43.
12. Štepánková K, Chudíčková M, Šimková Z, Martinez-Varea N, Kubinová Š, Urdzíková LM, et al. Low oral dose of 4-methylumbelliferone reduces glial scar but is insufficient to induce functional recovery after spinal cord injury. Scientific Reports. 2023;13(1):19183.
13. Nagy N, Kaber G, Haddock NL, Hargil A, Rajadas J, Malhotra SV, et al. The pharmacokinetics and pharmacodynamics of 4-methylumbelliferone and its glucuronide metabolite in mice. Hyaluronan: Structure, Biology and Biotechnology: Springer; 2023. p. 161-88.

14. Onufriev MV, Moiseeva YV, Zhanina MY, Lazareva NA, Gulyaeva NV. A comparative study of Koizumi and Longa methods of intraluminal filament middle cerebral artery occlusion in rats: early corticosterone and inflammatory response in the hippocampus and frontal cortex. International Journal of Molecular Sciences. 2021;22(24):13544.
15. Gao Y, Ya B, Li X, Guo Y, Yin H. Myricitrin ameliorates cognitive deficits in MCAO cerebral stroke rats via histone acetylation-induced alterations of brain-derived neurotrophic factor. Molecular and Cellular Biochemistry. 2021;476:609-17.
16. Zhan Y, Li M-Z, Yang L, Feng X-F, Lei J-F, Zhang N, et al. The three-phase enriched environment paradigm promotes neurovascular restorative and prevents learning impairment after ischemic stroke in rats. Neurobiology of Disease. 2020;146:105091.
17. Dubisova J, Burianova JS, Svobodova L, Makovicky P, Martinez-Varea N, Cimpean A, et al. Oral treatment of 4-methylumbelliferone reduced perineuronal nets and improved recognition memory in mice. Brain Research Bulletin. 2022;181:144-56.
18. Moradi A, Aslani MR, Mirshekari Jahangiri H, Naderi N, Aboutaleb N. Protective effects of 4-methylumbelliferone on myocardial ischemia/reperfusion injury in rats through inhibition of oxidative stress and downregulation of TLR4/NF-κB/NLRP3 signaling pathway. Naunyn-Schmiedeberg's Archives of Pharmacology. 2024:1-13.
19. Al Qteishat A, Gaffney JJ, Krupinski J, Slevin M. Hyaluronan expression following middle cerebral artery occlusion in the rat. Neuroreport. 2006;17(11):1111-4.
20. Hathcock KS, Hirano H, Murakami S, Hodes RJ. CD44 expression on activated B cells. Differential capacity for CD44-dependent binding to hyaluronic acid. Journal of immunology (Baltimore, Md: 1950). 1993;151(12):6712-22.
21. Kuipers HF, Rieck M, Gurevich I, Nagy N, Butte MJ, Negrin RS, et al. Hyaluronan synthesis is necessary for autoreactive T-cell trafficking, activation, and Th1 polarization. Proceedings of the National Academy of Sciences. 2016;113(5):1339-44.
22. Grandoch M, Bollyky PL, Fischer JW. Hyaluronan: a master switch between vascular homeostasis and inflammation. Circulation Research. 2018;122(10):1341-3.