

Isorhamnetin improves cognitive impairment of Alzheimer's disease through Sirt1/AKT/ERK/CREB signaling pathway

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Research Article

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Abstract

Isorhamnetin (IH), a flavonoid, has extensive pharmacological activities including anti-inflammation and antioxidation. However, the effects of IH in Alzheimer's disease (AD) are not very clear. This study investigates the effect and mechanism of IH in the pathological process of AD. Four-month-APP/PS1 mice were treated with IH in 50 mg/kg/d for 3 months. Morris water maze and immunofluorescence staining were used to detect whether IH could improve AD pathology. The mechanisms of IH improving AD pathology were detected by Western blot. The results indicated that IH improved the memory impairment of APP/PS1 mice by decreasing the time of escape latency as well as increasing the times of crossing the platform. Moreover, IH significantly reduced senile plaques deposition in the brain and increased the expression of ADAM10 in APP/PS1 mice through activating Sirt1/AKT/ERK/CREB pathway. Furthermore, IH reduced ROS formation, GP91 expression, upregulated the expression of SOD1 and Nrf2. Moreover, IH inhibited astrocytes activation and pro-inflammatory cytokine IL-1 β and TNF- α release. In conclusion, IH improved cognitive function, inhibited oxidative stress and inflammation response in AD. Therefore, we suggest that IH may be considered as a potential drug for AD treatment.

Introduction

Alzheimer's disease(AD) is a neurodegenerative disorder with insidious onset and slow progression and its incidence rate is increasing and the medical burden is becoming more and more serious.[1–2]. However, there are few drugs to treat AD in clinical. So far, only five drugs have been approved for AD and the role of these drugs is to control symptoms rather than change the process of the disease [2]. The main reason is that the pathogenesis of AD is not clear. At present, the amyloid hypothesis is considered to be one of the major pathogenesis of AD. Amyloid-beta (A β) are formed by amyloid precursor protein (APP) via sequential proteolytic cleavages via β -secretase (BACE1) and γ -secretase. A β can lead to synaptic damage and neuron loss, and ultimately to the pathological hallmarks of AD [2]. Therefore, inhibiting A β production can improve the pathological process of AD. The pathway of APP cleaved by the α -secretase A Disintegrin And Metalloprotease 10 (ADAM10) releases the soluble portion (sA β PP α) and prevents A β generation [3]. New treatments and preventive interventions targeting ADAM10 regulation for AD are necessary [3].

Isorhamnetin (IH) is one of the most important active components in the fruits of Hippophae rhamnoides L. and the leaves of Ginkgo biloba L., which has extensive pharmacological activities [4]. Previous studies showed that IH had the protection of cardiovascular and cerebrovascular, anti-tumor, anti-inflammatory, anti-oxidation, etc [4]. It has been showed that IH protected human RPE cells from oxidative stressinduced cell death, and this effect was associated with activation of the PI3K/Akt signaling pathway [5]. IH can also significantly prevent high-fat and high fructose diet (HFFD)-induced cognitive impairments [6]. Furthermore, IH inhibited HFFD-induced microglial overactivation and inflammatory cytokines released [6]. In addition, IH relieved the high glucose (HG)-induced oxygen-glucose deprivation and reoxygenation (OGD/R)-induced apoptosis, inflammatory response, and oxidative stress of HT22 cells. [7]. Recent research findings that IH treatment promotes functional recovery in rats induced by spinal cord injury

through inhibiting oxidative stress and modulating M1/M2 macrophage polarization [8]. The findings of the above studies suggested that isorhamnetin may have potential benefits in antioxidative effect and treatment of oxidative damage diseases. However, the effect of isorhamnetin on AD is not clear. Therefore, in this study, we investigated whether IH improved cognitive impairment and its mechanism by long-term treatment of AD model mice.

Materials And Methods

Animals and treatment

Four-month APP/PS1 transgenic male mice were randomly assigned into two groups: vehicle and IH groups. In IH group, IH drug was injected intraperitoneally once a day for 3 months at a dose of 50 mg/kg/d. The vehicle group was given the same amount of solvent. Mouse were kept the standardized conditions. All experimental procedures performed using animals were approved by the Laboratory of Animal Ethical Committee of China Medical University.

Behavior Tests

Morris water maze was to detect the behavior of APP/PS1mice after 3 months treatment. First, the mice were trained on the visual platform for two days, and then conducted the hidden platform experiment for five days to record the latency time of mice finding the platform by the water maze system (ZH0065; Zhenghua Bioequipment, China). On the eighth day, the platform was removed and the number of mice crossing the platform was recorded.

Tissue preparation

The mice were anesthetized and perfused with PBS. After that, the brains were quickly collected on ice, and one hemisphere was frozen and stored at -80°C, and the other was immersion-fixed in 4% paraformaldehyde for the histological study.

Reactive oxygen species (ROS) assay

Fresh tissue of the brain was prepared cell suspension and then centrifugation. Cell precipitation was resuspended with diluted DCFH-DA and incubated at room temperature for 30min. After washing with PBS for three times, and the fluorescence intensity of ROS was detected by Flow cytometry.

Measurement of oxidative stress related enzymes activities

Brain tissue was homogenized with cold PBS in the ratio of 1:10 at 4 °C and then centrifuge 12000g at 4 °C for 3–5 minutes. The supernatant protein concentration was measured by BCA protein assay kit and then the activities of SOD and GSH were detected by assay kit according to the manufacturer's instructions (Najing Jiancheng).

Immunofluorescence staining

The frozen sections were blocked with 5% goat serum for 30 min and incubated with anti-GAAPP antibody and anti-A β overnight at 4°C. The sections were washed with PBS for 3 times and then incubated with Alexa Fluor 594- or Alex Fluor 488-conjugated secondary antibodies for 1.5 h. Images were detected by using the laser scanning confocal microscope (TCS SP8, Leica, Germany).

Western blot

The brain tissues were homogenized with RIPA lysis buffer including protease and phosphatase inhibitor on ice for 3 h. After centrifugation, the protein concentration was determined through BCA protein assay kit. The protein samples were loaded according to the same amount of protein and were separated by 10% SDS-PAGE gels. After transfection, PVDF membrane was blocked with 5% BSA for 30 min and incubated with different primary antibodies overnight at 4°C. The next day, membrane were washed 3 times with TBST and then incubated with HRP-conjugated secondary antibody for 1.5 h, and finally were detected using ECL.

Results

IH treatment significantly improve cognitive impairment in APP/PS1 mice

To illustrate whether IH improves cognitive impairment, APP/PS1 mice were subjected to Morris water maze. The results showed that after IH treatment, the latency time for mice to find the platform was significantly reduced (Fig 1A). The number of mice crossing the platform was significantly more than that of the vehicle group (Fig 1B). The results suggested that IH could significantly improve the cognitive impairment of mice.

IH treatment alleviated A β deposition in APP/PS1 mice

In order to explore the mechanism of IH improving behavior, we detected the generation of senile plaques by immunofluorescence. The results showed that the senile plaques in the brain of mice decreased significantly after three months of IH treatment (Fig2).

IH up-regulated the expression of ADAM10

A β is produced by the cleavage of APP through amyloid pathway, BACE1 and γ -secretase are the main cleavage enzymes. The results showed that IH had no effect on the expression of BACE1 and γ -secretase (Fig 3A-B). However, ADAM10, as the main enzyme in the non-amyloid pathway, our results show that IH can significantly up regulate its expression (Fig 3A-B) and then reduce the production of A β .

IH inhibited oxidative stress in APP/PS1 mice

Oxidative stress is considered to be a key factor in the pathophysiology of AD. Excessive production of reactive oxygen species (ROS) and disorder of antioxidant capacity led to subsequent oxidative damage [9]. To illustrate the effects of IH on oxidative stress, we investigated the levels of ROS, SOD and GSH in brain tissue of mice. The results showed that the fluorescence intensity of DCFH-DA was significantly

decreased after IH treatment (Fig 4A). The level of SOD and GSH was enhanced compared to the vehicle group (Fig 4B).

GP91 has been recognized as biomarkers of oxidative stress [10-11]. In this study, IH significantly decreased the levels of GP91 (Figure 4 C). Taken together, these results illustrated that IH could alleviate oxidative stress in APP/PS1 mice.

A large number of studies have shown that the transcriptional factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) plays a vital defensive role in the antioxidant response of the brain. Nrf2 activation promotes the expression of several antioxidant enzymes that exert cytoprotective effects against oxidative damage [12]. In our paper, we found that IH increased the expression of Nrf2 to against oxidative stress (Fig 4C).

Isorhamnetin reduced inflammatory response in APP/PS1 mice

Inflammation plays an important role in the pathogenesis of AD. In order to confirm whether IH inhibits inflammatory response in AD, we detected the release of inflammatory factors and astrocytes activation. The results found that after IH treatment, the expression of TNF- α and IL-1 β were decreased (Fig 5A-B). Western blotting and immunofluorescence results showed that IH could inhibit astrocytes activation induced by A β (Fig 5B).

Isorhamnetin activated Sirt1/AKT/ERK/CREB signaling pathways

Previous study showed that CREB is the promoter of ADAM10 and up-regulating CREB can promote the expression of ADAM10. ERK is the upstream of CREB, it can be activated by AKT and its activation can promote the phosphorylation of CREB. Our results showed the IH significantly activated AKT and promoted phosphorylation level of ERK and CREB (Fig 6A-B). Notably, Sirt1 expression activated the Akt signaling pathway, and IH can increase the Sirt1 expression (Fig 6A-B).

Discussion

The results of this study show that IH, a flavonoid, can significantly improve the cognitive impairment of APP/PS1 transgenic mice, reduce the deposition of senile plaques in the brain, up-regulate the expression of ADAM10 by activating SIRT1/Akt /ERK/CREB signal pathway, and promoting the non-amyloid pathway of APP. And IH also reduced the production of ROS in the brain, increased the expression and activity of antioxidant enzymes SOD1, GSH and the expression of Nrf2, inhibited inflammation, and improved the pathological process of AD.

A β is released from APP through sequential cleavages by BACE-1 and γ -secretase. In nonamyloidogenic pathway, APP can be cleaved by α -secretase and γ -secretase to inhibit A β production [13]. In neurons, ADAM10 (metalloprotease) is considered the major α -secretases [13]. Up-regulating ADAM10 can suppress A β production [14], suggesting that ADAM10 may serve as a potential target in arresting AD. Previous study showed that CREB as ADAM10 promoter was induced could increase the expression of ADAM10 to preclude A β production [15]. The ERK-CREB signal pathway has been studied for its role in AD

[15]. Previous studies have shown that the neuroprotective effect of AD may be caused by CREB activation mediated by ERK activation, which promotes the expression of ADAM10 [15]. Inhibiting ERK could lead to decrease phosphorylation level of CREB. In addition, artemisinin regulated the protective effects against A β 1-42-induced damage by ERK/CREB pathway [16]. According to these results, IH plays the protective effect in AD via promoting ADAM10 by activating ERK/CREB pathway.

Studies suggest that activation of phosphoinositide AKT may protect against neuronal cell death in AD [17]. It has been found that activating the AKT signaling pathway could upregulate HO-1 expression and enhance nuclear translocation of Nrf2 [18]. Upregulated phosphatidylinositol 3-kinase (PI3K)/AKT signaling decreased A β levels and A β deposition in brain and ameliorated toxicity in A β -treated primary neuronal culture [19]. Moreover, AKT as an upstream kinase regulating ERK activation and AKT inhibitor or AKT siRNA could decrease the phosphorylation of ERK [20], suggesting that ERK phosphorylation is dependent on AKT. Sirt1, one of the sirtuin family of NAD(+)-dependent deacetylases, has recently been shown to attenuate amyloidogenic processing of amyloid- β protein precursor (APP) in AD by increasing α -secretase production and activity [21]. Sirt1 expression activated the AKT signaling pathway [22]. In our study, we found IH could activate AKT via increasing Sirt1 expression and then lead to upregulate the ERK phosphorylation.

Oxidative stress, considered to be a key factor in the pathophysiology of stroke, is caused by the excessive production of reactive oxygen species (ROS) and the disorder of antioxidant capacity [23]. GP91 had been recognized as biomarkers of oxidative stress [10–11]. Our results showed that IH treatment could reverse the up-regulation of ROS and GP91 expression. Moreover, the activities and expression of antioxidant enzymes SOD and GSH were increased. Nuclear factor erythroid-related factor 2 (Nrf2) has been implicated in antioxidant defense processes and plays a key role against oxidative stress [24]. Previous studies have shown that up-regulation Nrf2 can alleviate H2O2-induced oxidative stress [24]. It has shown that activation of Nrf2 restored the decreased activities of GSH, CATs and SODs [25–27]. Our results illustrated that IH treatment enhanced the Nrf2 expression, thereby improving the activity of antioxidant enzymes.

Inflammation is also associated with AD and that could have a role in contributing to the pathogenesis of AD [28]. Astrocytes are key regulators of inflammatory responses in the central nervous system [29]. Astrocyte activity may exacerbate inflammatory reactions by releasing numerous molecules, including interleukins, tumor necrosis factor alpha, etc [30]. Our results show that IH can inhibit the activation of astrocytes and the release of inflammatory factors and reduce the inflammatory response.

In conclusion, IH promotes the expression of ADAM10 by activating SIRT1/AKT/ERK/CREB signaling pathway, thereby reducing the deposition of senile plaques. In addition, IH can also inhibit oxidative stress and inflammatory response. finally, improve the cognitive impairment of AD.

Figure legends

Figure 1. IH improves cognitive impairment of APP/PS1 mice. Four-month-APP/PS1 mice were treated with IH (i.p., 50 mg/kg/d) for 3 months. Morris water maze was used to test cognitive ability including 2 days of visible platform training, 5 days of hidden platform testing, and a probe trial. (A) In the visible platform and the hidden platform, the escape latency time of mice found the platform. (B) In the probe trial, the times of crossing the platform were recorded. Data were presented as the mean ± SD; n = 6, * P < 0.05; ** P < 0.01.

Figure 2. IH inhibites senile plaque deposition in APP/PS1 mice. Four-month-old APP/PS1 mice were treated with IH for 3 months. A β plaque in the cortex and hippocampus of APP/PS1 mice were detected by immunofluorescent.

Figure 3. IH upregulates the expression of ADAM10 in APP/PS1 mice. Four-month-old APP/PS1 mice were treated with IH for 3 months. Western blot detect the expression levels of ADAM10, BACE1 and the subunits of γ -secretase, including NCT, PS2 and APH-1. Data were presented as the mean ± SD; n = 6, * P < 0.05; ** P < 0.01; *** P < 0.001.

Figure 4. IH reduce the oxidative stress in APP/PS1 mice. Four-month-old APP/PS1 mice were treated with IH for 3 months. (A,) Western blot detect the expression levels of SOD1, Nrf2 and GP91 in the cortex. (B) ROS production in the cortex was detected with the dichlorofluorescein diacetate probe. Data were presented as the mean ± SD; n = 6, * P < 0.05; ** P < 0.01.

Figure 5. IH alleviated the neuroinflammatory response in APP/PS1 mice. Four-month-old APP/PS1 mice were treated with IH for 3 months. (A-B) Immunoblot analysis showed the expression levels of IL-1 β and TNF- α . (C) IH treatment suppressed the activation of astrocytes around the A β plaque. Data were presented as the mean ± SD; n = 6, ** P < 0.01.

Figure 6. IH activated the Sirt1/AKT/ERK/CREB signaling pathways in the APP/PS1 mice. Four-month-old APP/PS1 mice were treated with IH for 3 months. (A-B) Immunoblot analysis showed the expression levels of Sirt1, p-AKT, AKT, p-ERK, ERK, p-CREB and CREB. Data were presented as the mean ± SD; n = 6, * P < 0.05; * P < 0.05; ** P < 0.01

Ethics approval

All experimental procedures performed using animals were approved by the Laboratory of Animal Ethical Committee of China Medical University.

Declarations

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Consent to Participate Not applicable

Consent to Publication Not applicable

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Authors Contributions

Ying Li and Sha Sha designed research and wrote the paper. Zhang Rong Wei participated in data analysis and Figures making. All authors have read and approved the last manuscript.

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Figures

Figure 1

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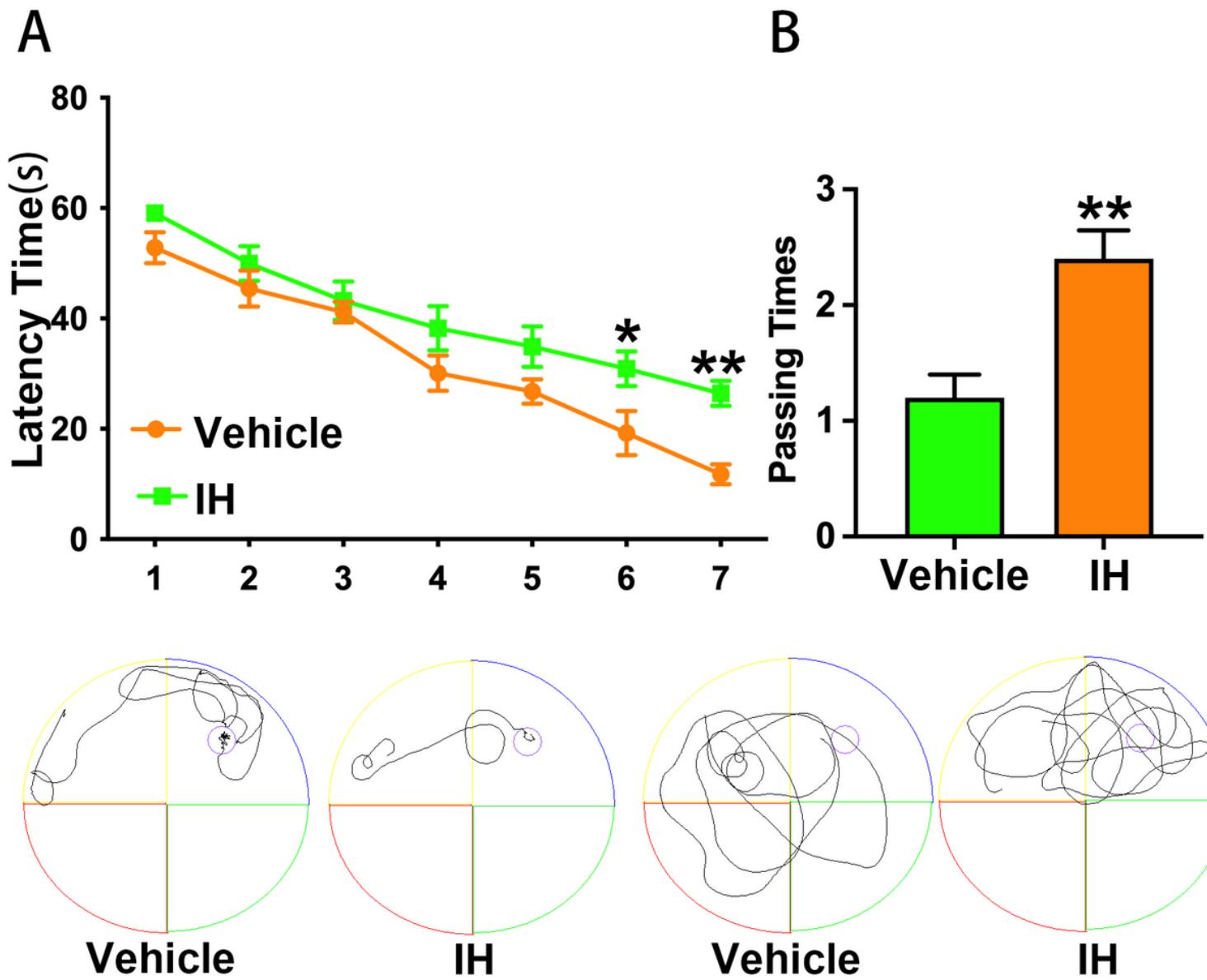


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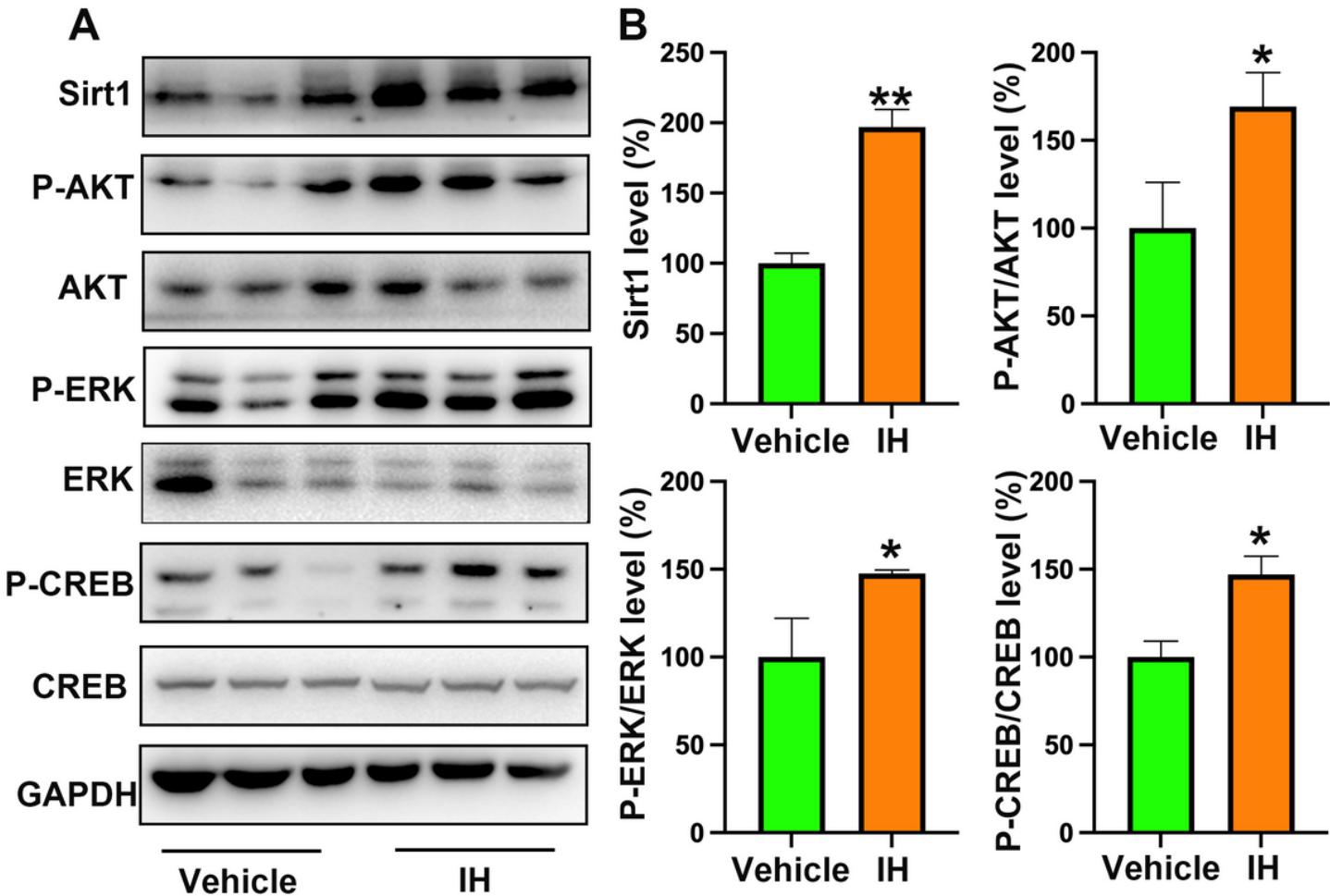


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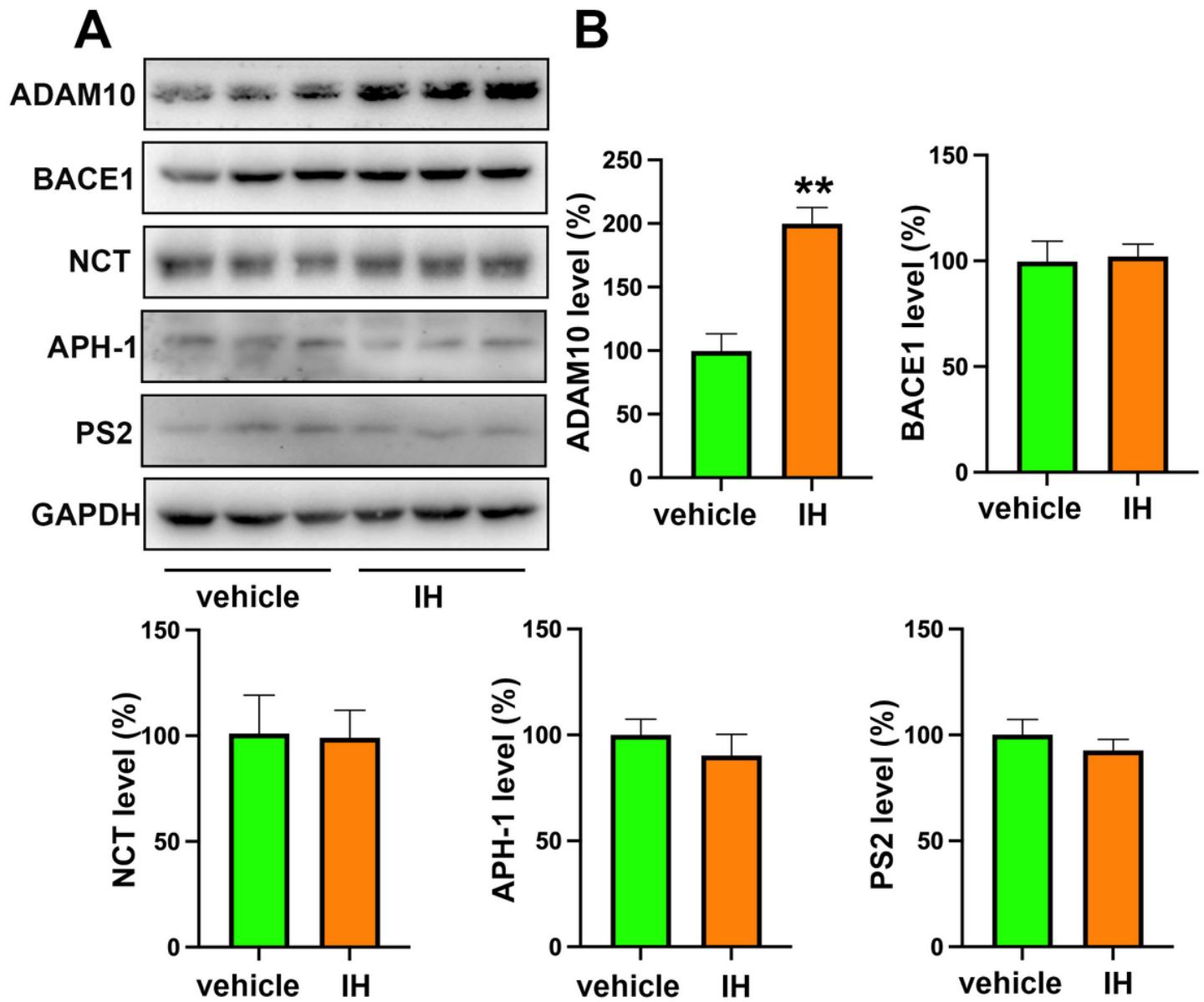


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