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ADAM17 Inhibition Protects Cognition in Intermittent Hypoxia: The Role of TREM2

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Purpose: The triggering receptor expressed on myeloid cells 2 (TREM2) is a new therapeutic target in Alzheimer's disease. However, its role in obstructive sleep apnea (OSA)-related cognitive impairment is still unclear. This study aimed to investigate the effect and regulatory mechanism of TREM2 on cognitive impairment related to OSA.

Methods: Since intermittent hypoxia (IH) is the primary pathophysiologic characteristic of OSA, we conducted IH animal and BV2 cell model to investigate the mechanism. *Trem2* knockdown and *Trem2* overexpression cells were created by Lentivirus transfection. A disintegrin and metalloprotease 17 (ADAM17) is the primary enzyme for TREM2 shedding, we used TAPI-1 to inhibit its activity. Morris water maze, Nissl staining, real-time PCR, immunofluorescence, Western blotting, fluorometric assay kit, and enzyme-linked immunosorbent assay were used to explore the molecular mechanism.

Results: The TREM2 levels were decreased in BV2 cells exposed to IH for 24 hours. IH elevated the levels of IL-1β, TNF-α and CD86 in BV2 cells, as well as the levels of p-Tau in conditioned media-cultured HT-22 cells. Conversely, IH reduced the levels of IL-10 and CD206 in BV2 cells. However, these effects were exacerbated in BV2 cells with *Trem2* knockdown, whereas they were mitigated in those with *Trem2* overexpression. Additionally, the ADAM17 activity and soluble TREM2 (sTREM2) levels were increased in BV2 cells subjected to IH. Treatment with TAPI-1, suppressed ADAM17 activity and restored TREM2 expression both in vitro and in vivo. Inhibition of ADAM17 led to a reduction in the expression of CD86, IL-1β, TNF-α and p-Tau levels, while enhancing the expression of CD206, IL10 and cognitive functions.

Conclusion: TREM2 played a protective role in IH-induced neuroinflammation and neuronal injury by promoting microglia M2 polarization. IH caused excessive activation of ADAM17 and resulted in augmented degradation of TREM2. Restoring TREM2 expression by inhibiting ADAM17 indicates a potentially promising therapeutic strategy for cognitive impairment in OSA.

Keywords: obstructive sleep apnea, intermittent hypoxia, TREM2, ADAM17, cognitive impairment, neuroinflammation

Introduction

Cognitive impairment is a prevalent complication of obstructive sleep apnea (OSA), manifesting as the decline of attention, memory, executive function, visual space, and exerting a detrimental impact on life quality and disease prognosis.[1–4] In view of unknown etiology and no effective medicines, it posed a great challenge on medical and social burden. The studies conducted by Sun et al and Liu et al observed a significant relationship between proinflammatory cytokines and neurocognitive performance in OSA patients.[5,6] Gozal et al found that intermittent hypoxia (IH), a prominent feature of OSA, could induce cognitive impairment in rats.[7] Subsequent research indicated that IH could trigger microglial activation and release of pro-inflammatory factors, ultimately resulting in neuroinflammation and neuronal damage.[8,9] Therefore, elucidating the mechanism of inflammation and attenuating the inflammatory response may effectively mitigate cognitive impairment in patients with OSA.

Triggering receptor expressed on myeloid cells 2 (TREM2) is an immunoglobulin superfamily transmembrane protein, primarily expressed on microglial cells in the central nervous system, which plays a protective role in neuroinflammation.[10,11] The overexpression of *Trem2* has been reported to facilitate microglia M2 polarization and attenuate neuroinflammation induced by intracerebral or subarachnoid hemorrhage, beta-amyloid, and lipopolysaccharide.[12–15] Therefore, TREM2 is supposed as a potential therapeutic target for some neurodegenerative diseases. Wu et al reported that the expression of TREM2 in the brain tissue was influenced by ischemia and hypoxia,[16]

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however, little was known about the effect of TREM2 on IH-induced neuroinflammation and cognitive impairment. It has been reported that a disintegrin and metalloprotease 17 (ADAM17) is the main proteolytic enzyme that cleaves the extracellular domain of TREM2 into soluble TREM2 (sTREM2) at its His157 residue within the stalk region.[17] Hypoxia has been shown to enhance the enzymatic activity of ADAM17.[18,19] Our previous investigation has identified that the serum sTREM2 increases in OSA patients with mild cognitive impairment (MCI).[20] Therefore, we speculated that IH promoted ADAM17 overactivation and TREM2 proteolysis, and then caused neuroinflammation and neuronal injury. With BV2 cells and mice models, this study aimed to investigate the effect of TREM2 on IH-induced cognitive impairment and its underlying modulatory mechanism, thereby providing novel insights for therapeutic strategy.

Materials and Methods

Animals, IH Model Development and Drug Treatment

The male C57BL/6J mice (6–8 weeks old, weighing 25 ± 2 g) were obtained from the Animal Experimental Center at China Medical University. A total of 30 mice were randomly divided into the following groups: room air group (RA group, n=10), IH for eight weeks group (IH group, n=10), and IH+TAPI-1 group (n=10). We estimated the sample size before the data analysis based on the results of the pre-experiment, and the target power of the design was 80%. The mice in the IH group were exposed to oxygen levels oscillating between 21% and 6% with a period of 180s for a cycle (nitrogen was rapidly filled in the first 40s to reduce the oxygen concentration in the hypoxic chamber to 6% and maintain for 50s, and then oxygen was rapidly filled in the next 40s to increase the oxygen concentration in the hypoxic chamber to 21% and maintain for 50s) for 8h per day (from 8:00 to 16:00) with a duration of 8 weeks by using OxyCycler software (OxyCycler Model A84, USA). The mice in the RA group were placed under normal air conditions over the same period. The mice in the TAPI-1 group additionally received intraperitoneal injections of TAPI-1 (an ADAM17 inhibitor, Selleckchem, USA) at a dosage of 10 mg/kg every three days for six weeks after two weeks of IH exposure.[21] All the experimental protocols followed the ARRIVE guidelines and were approved by the Ethics Committee of China Medical University following the guidelines outlined in the National Institutes of Health Guide regarding the care and use of experimental animals (KT20240116).

Cells, IH Model Development and Drug Treatment

BV2 cells, derived from murine microglia, was obtained from the Chinese Academy of Medical Sciences. They were placed in a chamber (OxyCycler) with a 60–70% confluence with the oxygen concentration altered between 1% to 21% with a period of 30min for a cycle (In the first 5 min, nitrogen was filled rapidly to make the oxygen concentration in the hypoxic chamber decrease and maintain at 1% for 10min. In the next 5 min, oxygen was filled rapidly to make the oxygen concentration rise and maintain at 21% for 10 min) via injection of oxygen or nitrogen. The microglial cells were collected for Western blotting analysis at 3, 6, 9, 12, and 24 h of IH exposure.

Trem2 knockdown and *Trem2* overexpression on BV2 cells were created through lentivirus transfection following the manufacturer's protocol (Genechem). Briefly, the cells were seeded in six-well plates and cultured overnight before transfection. Subsequently, they were transfected with LV- *Trem2*-RNAi / LV-*Trem2*-OE / LV-NC and cultured with DMEM without FBS for 16 h. After 72 h of transfection, stable strains were obtained by selecting cells with puromycin (4 μg/mL, Genechem). Cells with correct genetic identification were cryopreserved and subjected to subsequent experiments.

TAPI-1 was dissolved in DMSO to a stock solution of 1 mM concentration. When BV2 cells reached a confluency level of 60–70%, they were pre-treated with 100nM / 500nM / 1000nM TAPI-1 for 30 min and then exposed to IH for 24h. Finally, immunofluorescence and RT-PCR were conducted on the collected samples.

Conditioned Culture of HT-22 Cells

Mouse hippocampal HT-22 cells were acquired from iCell bioscience. The supernatant medium from microglia cells that had been subjected to various treatments was collected and mixed with the fresh complete medium at a 1:1 ratio,

resulting in a conditioned medium. When HT-22 cells reached a confluency level of 60–70%, they were cultured in the conditioned medium for 24 h and then harvested for p-Tau expression analysis.

Morris Water Maze Test

Morris water maze was employed to evaluate the spatial learning and memory abilities of mice as previously described method.[22] It consisted of a circular pool (1.5 m in diameter and 50 cm in height) filled with non-transparent white water up to a depth of 30 cm. A platform was positioned 1 cm below the water surface within one of the quadrants. During five consecutive days of training trials, mice were introduced into the pool from four different quadrants sequentially. The time for finding the platform was recorded as the escape latency. In case mice failed to find the platform within 120s, they were gently guided toward it. 24 hours after the completion of training trials, mice were subjected to a probe trial lasting for 120s without the presence of any platform. Parameters such as the percentage of time spent in the target quadrant, the number of crossings over the previous location of the platform, and average swimming speed were recorded.

Nissl and Immunofluorescence Staining

After the behavior test, mice (4/group) were anesthetized with pentobarbital at a dosage of 0.1mL/10g. The whole brain tissues were immediately extracted and divided into hemispheres, which were then fixed in 4% paraformaldehyde at 4 °C for 24–48 hours. The right hemispheres underwent dehydration, paraffin embedding, and subsequent sectioning into 4 μm thick slices. Nissl staining was performed for pathological analysis, and images were captured by a light microscope (Olympus, Japan). The left hemispheres were equilibrated in 30% sucrose before obtaining 25 μm sagittal slices with a freezing microtome. These hippocampal sections along with BV2 cell slide climbing underwent three washes with PBS followed by antigen blocking, and overnight incubation with CD86 antibody (Abcam, 1:100) or CD206 antibody (Abcam, 1:100) at 4°C. After incubating with a secondary antibody and DAPI at room temperature, all the slices were captured under a microscope. ImageJ software was used for result analysis.

Western Blotting

Proteins from the hippocampal tissues or BV2 cells were separated by SDS-PAGE, transferred onto PVDF membranes, blocked with 5% milk for 1 hour, and subsequently incubated with specific primary antibodies against TREM2 (CST, 1:1000), p-Tau (Abcam, 1:1000), β-actin (Affinity, 1:2000) at 4°C overnight. Immunoreactive bands were detected by ECL (Uelandy, China) after incubation for an additional hour with a secondary antibody (Affinity, 1:5000) at room temperature. ImageJ software was used for analysis and the relative protein expressions were normalized to β-actin.

RT-PCR

The hippocampal tissues of mice or BV2 cells were subjected to Trizol (Accurate Biology, China) extraction for total RNA isolation. The mRNA expression of IL-10, TNF-α, and IL-1β were quantified by using SYBR Green Pro Taq HS (Accurate Biology, China) according to the manufacturer's instructions. The relative expressions were normalized to β-actin using the $2^{-\Delta\Delta CT}$ method. Primer sequences were listed in Table S1 obtained from Sangon Biotech (China).

ADAM17 Activity

The detection procedure for ADAM17 activity in the hippocampus and BV2 cells were as follows. The samples were homogenized in lysis buffer containing 1% Triton X-100 and centrifuged at 12000 g for 10 min at a temperature of 4°C. ADAM17 activity was measured using a fluorometric assay kit (AnaSpec, USA) according to the operation manual. Fluorescence intensity was monitored at an excitation/emission wavelength pair of 490nm/520nm. Finally, the enzyme activity per microgram of protein was calculated.

Enzyme-Linked Immunosorbent Assay (ELISA)

The hippocampus tissues were homogenized in PBS containing 1% cocktail and 1% phosphatase inhibitor, followed by centrifugation at 12000 *g* for 10 min at 4°C. The concentration of total protein was determined using a BCA kit and then diluted to a final concentration of 2.5 mg/mL. The cell supernatant was collected. The level of sTREM2 in the mouse

hippocampus and cell supernatant was quantified using a mouse sTREM2 ELISA kit (J&L, China) according to the manufacturer's instructions.

Statistical Analysis

Data were analyzed using SPSS 26.0 and presented as the mean ± SD. The comparison between two groups was evaluated using unpaired two-tailed Student's *t*-test, and comparisons among three or more groups were evaluated using one-way or two-way ANOVA. The relationship between the two biomarkers was examined by Pearson's correlation analysis. Differences were considered statistically significant at $P < 0.05$.

Results

TREM2 Played a Protective Role in IH-Induced Neuroinflammation and Neuronal Injury

BV2 microglia was used to assess the impact of IH on TREM2. After 24 hours of IH exposure, BV2 cells showed a decline in TREM2 levels (Figure 1A), an increase in pro-inflammatory factors (TNF-α and IL-1β), and a reduction in anti-inflammatory factor (IL-10) levels (Figure 1B–D). For further validation effect of TREM2, we subsequently

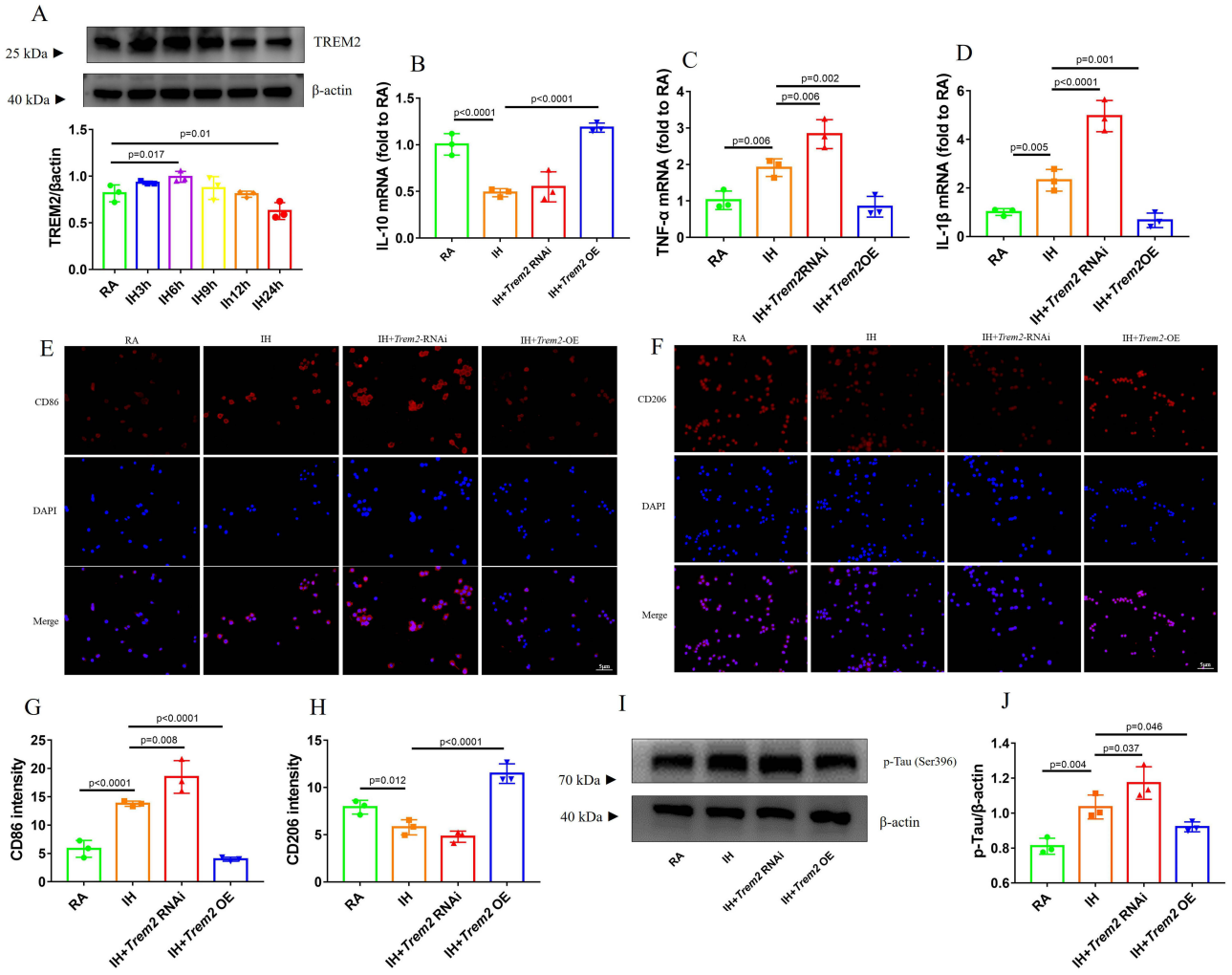

Figure 1 TREM2 played a protective role in IH-induced neuroinflammation and neuronal injury. (**A**) The representative Western blotting images and its quantified results of TREM2 in BV2 cells of different groups. (**B–D**) The RT-PCR results of IL-10 mRNA (**B**), TNF-α mRNA (**C**), and IL-1β mRNA (**D**) in BV2 cells of different groups. (**E–H**) The representative immunofluorescence images and its quantified results of CD 86 (**E** and **G**) and CD206 (**F** and **H**) in BV2 cells of different groups (original magnification, 200×). (**I-J**) The representative Western blotting images and its quantified results of p-Tau (Ser396) in HT-22 cells of different groups. Data were presented as means ± SDs.

established BV2 cell lines with *Trem2* knockdown (*Trem2*-RNAi) or *Trem2* overexpression (*Trem2*-OE) (Figure S1). When compared to the IH group, the *Trem2*-RNAi group presented a further heightened expression of pro-inflammatory factors, whereas the *Trem2*-OE group showed a reduction in pro-inflammatory factors and an increase in anti-inflammatory factors. These findings suggested that IH induced neuroinflammation by decreasing TREM2 expression.

It has been reported that neuroinflammation is related to M1/M2 polarization of microglia.[23] Therefore, we detected the expression of the M1 biomarker (CD86) and M2 biomarker (CD206) of BV2 cells by immunofluorescence (Figure 1E–H). Compared to the RA group, the IH group showed an increase in CD86 expression and a decrease in CD206 expression, indicating that IH promoted M1 polarization. Furthermore, compared to the IH group, BV2 cells of the *Trem2*-RNAi group presented a further elevated expression of CD86, while those of the *Trem2*-OE group showed a decreased expression of CD86 and an increased expression of CD206. These suggested that TREM2 took part in IH-induced neuroinflammation by modulating M1/M2 polarization of microglia.

To further analyze the influence of TREM2 on neuronal damage induced by IH, we cultivated mouse hippocampal neurons HT-22 in a conditioned medium with TREM2 treatment. As a crucial component in the axons of neurons, Tau plays a pivotal role in axonal transport and stability.[24] Phosphorylated Tau (p-Tau) impacts neuronal function and serves as a biomarker of cognitive impairment. Therefore, we assessed the expression of p-Tau via Western blotting as an indicator of neuronal damage. Our results demonstrated that IH induced a significant increase in p-Tau levels in neurons. When compared to the IH group, the *Trem2*-RNAi group exhibited a further increase while the *Trem2*-OE group showed a significant reduction of p-Tau expression (Figure 1I and J). These findings suggested that TREM2 could exhibit a neuroprotective effect against IH-associated neuronal damage.

Overactivation of ADAM17 Induced by IH Led to Increased Hydrolysis of TREM2

TREM2 can be hydrolyzed by ADAM17 into sTREM2 which can be detected in serum.[17] Our previous study indicated that patients with OSA exhibited an elevated level of serum sTREM2, particularly those with MCI.[20] Therefore, we investigated the level of sTREM2 and ADAM17 enzymatic activity in BV2 cells. The results showed that the sTREM2 level of the supernatant increased in the IH group compared to that in the RA group. Additionally, ADAM17 enzymatic activity increased when exposed to IH, especially in the IH24h group, while its mRNA level did not change (Figure 2A–C). Further Pearson correlation analysis revealed a positive correlation between ADAM17 activity and sTREM2 level (Figure 2D). To further confirm whether the decline of TREM2 during IH exposure was from the excessive activation of ADAM17 which led to increased protein hydrolysis, ADAM17 inhibitor TAPI-1 was used. We discovered a dose-dependent trend between TAPI-1 and ADAM17 enzymatic activity, so as to TAPI-1 and sTREM2 levels (Figure 2E and F). The expression of TREM2 was also restored with an increased dose of TAPI-1 (Figure 2G). These results revealed that IH exposure promoted TREM2 proteolysis and reduced the expression of full-length TREM2 on microglia by overactivation of ADAM17.

Inhibition of ADAM17 Activity Effectively Ameliorated Neuroinflammation and Neuronal Injury Induced by IH in vitro

We further investigated the effects of ADAM17 inhibition on neuroinflammation and neuronal injury induced by IH. The RT-PCR results revealed that compared to the IH group, the TAPI-1 group presented an increase in anti-inflammatory factor (IL-10) and a decrease in pro-inflammatory factors (TNF-α and IL-1β) (Figure 3A–C). Immunofluorescence analysis showed that TAPI-1 treatment resulted in CD86 reduction and CD206 increase (Figure 3D–G). Furthermore, we assessed the impact of ADAM17 inhibition on neuronal injury by using conditioned-culture neuronal cells. Our findings demonstrated that TAPI-1 treatment significantly decreased the expression of p-Tau (Figure 3H). Collectively, these results suggested that inhibition of ADAM17 activity promoted microglial M2 polarization, and alleviated neuroinflammation and neuronal injury induced by IH in vitro.

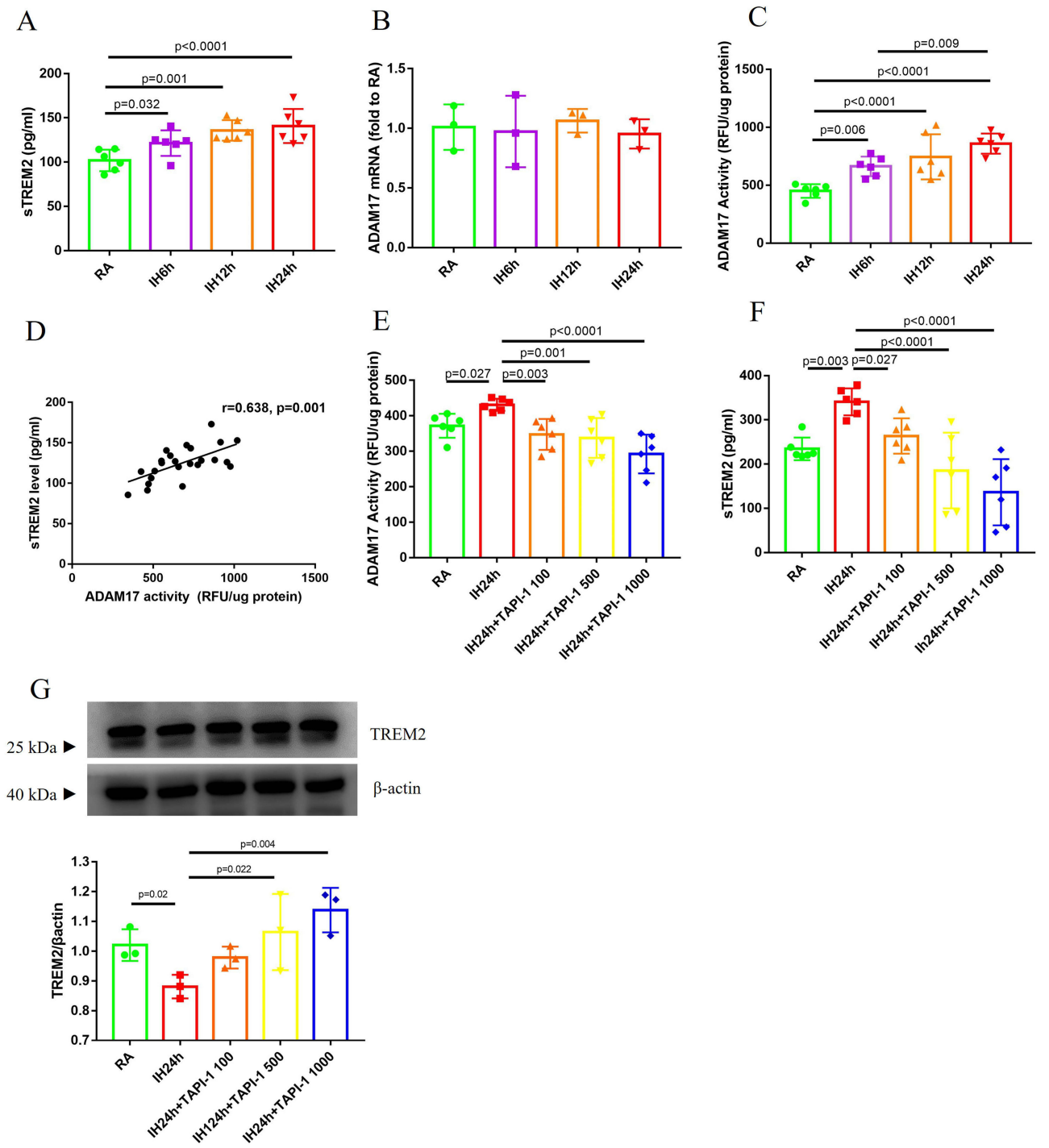

Figure 2 Overactivation of ADAM17 induced by IH increased the hydrolysis of TREM2. (**A**) The ELISA results of sTREM2 in the supernatant of BV2 cells of different groups. (**B**) The RT-PCR results of ADAM17 mRNA. (**C**) The results of ADAM17 activity in BV2 cells of different groups. (**D**) The Pearson correlation analysis between ADAM17 activity and sTREM2 level. (**E**) The results of ADAM17 activity in BV2 cells of different groups with TAPI-1 treatment. (**F**) The ELISA results of sTREM2 in the supernatant of BV2 cells of different groups with TAPI-1 treatment. (**G**) The representative Western blotting images and its quantified results of TREM2 in BV2 cells of different groups with TAPI-1 treatment. Data were presented as means ± SDs.

Inhibition of ADAM17 Activity Reduced the Hydrolysis of TREM2 in IH-Exposed Mice

To further elucidate the change of TREM2 and the role of ADAM17 on TREM2 shedding during IH in vivo, we constructed the IH+TAPI-1 group, in which mice received an intraperitoneal administration of ADAM17 inhibitor (TAPI-1). The results showed that in the IH condition, the expression of full-length TREM2 in the hippocampus

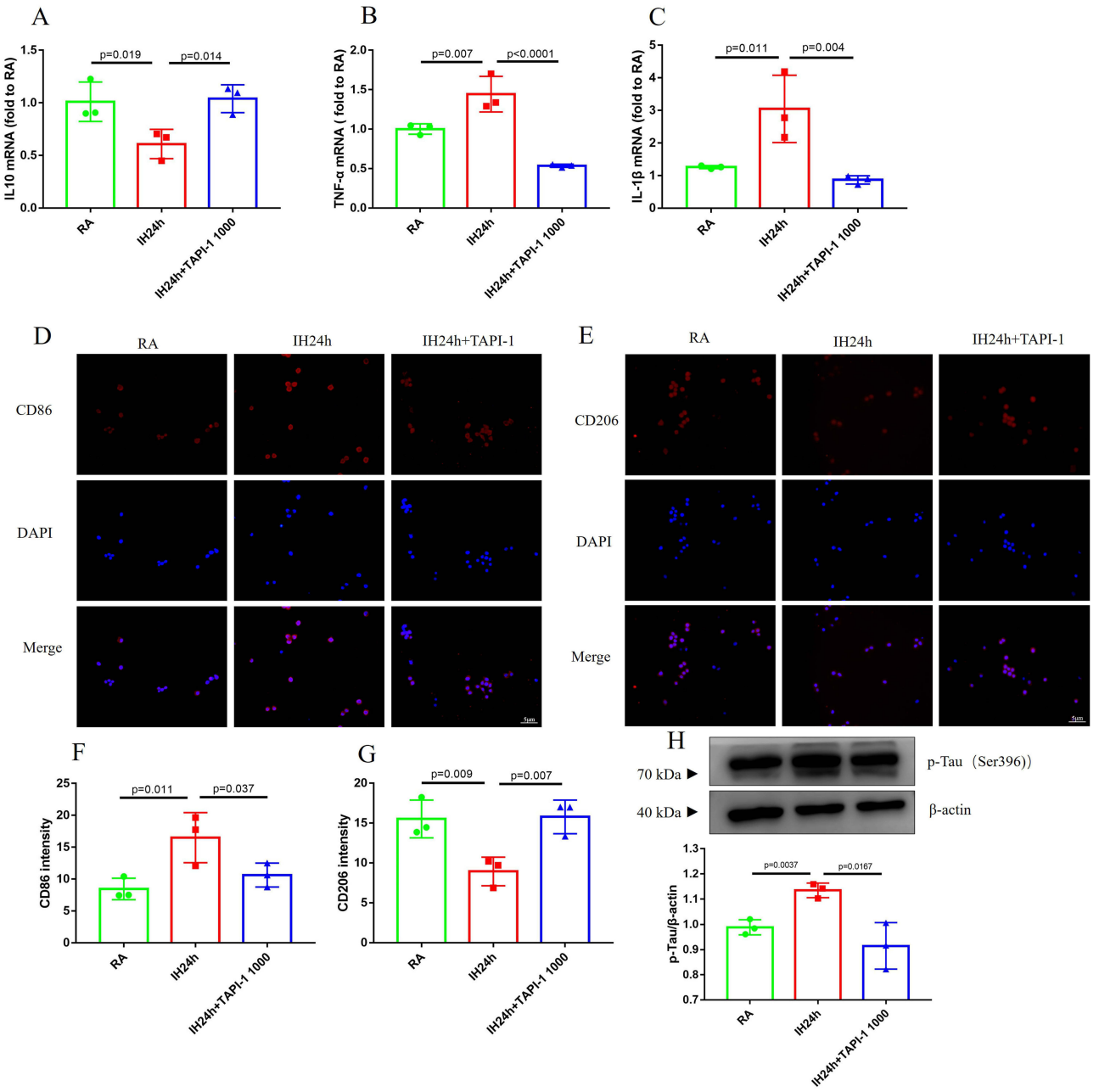

Figure 3 Inhibition of ADAM17 activity effectively ameliorated neuroinflammation and neuronal injury induced by IH in vitro. (**A-C**) The RT-PCR results of IL-10 mRNA (**A**), TNF-α mRNA (**B**), and IL-1β mRNA (**C**) in BV2 cells of different groups with TAPI-1 treatment. (**D–G**) The representative immunofluorescence images and its quantified results of CD 86 (**D** and **F**) and CD206 (**E** and **G**) in BV2 cells of different groups with TAPI-1 treatment (original magnification, 200×). (**H**) The representative Western blotting images and its quantified results of p-Tau (Ser396) in HT-22 cells of different groups. Data were presented as means ± SDs.

decreased along with an increase in ADAM17 activity and sTREM2 level, whereas TAPI-1 treatment reduced the levels of ADAM17 activity and sTREM2 and restored the expression of TREM2 (Figure 4A–F). However, the mRNA level of ADAM17 was not influenced (Figure 4G).

The Inhibition of ADAM17 Activity Alleviated the Neuroinflammation in the Hippocampus of IH-Exposed Mice

Compared to the RA group, the IH group showed increased CD86 and decreased CD206 in the hippocampus of mice. These alterations were reversed after TAPI-1 treatment (Figure 5A–D). Subsequently, we assessed the inflammatory state

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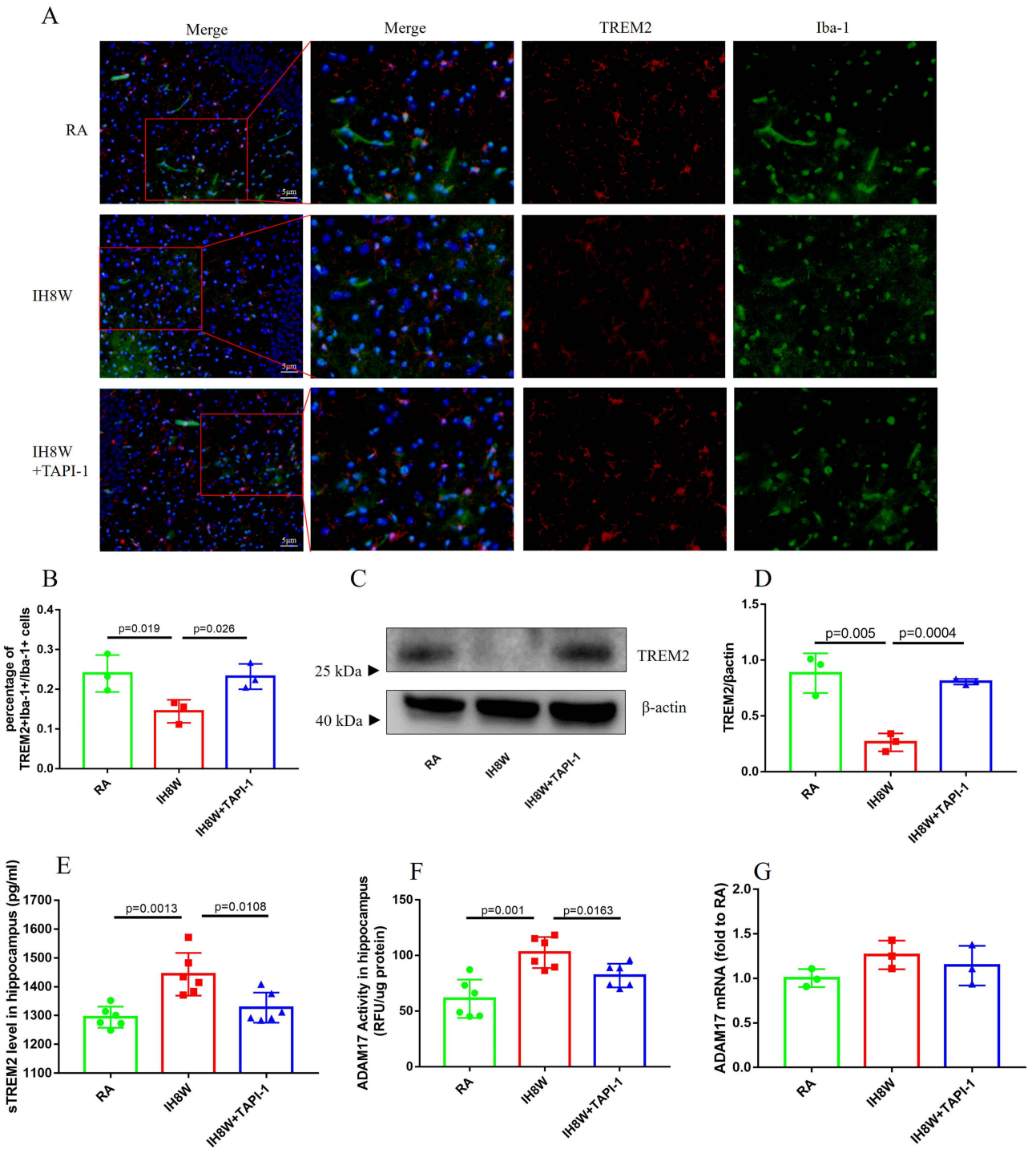

Figure 4 ADAM17 inhibitor reduced the hydrolysis of TREM2 in the hippocampus of IH-exposed mice. (**A** and **B**) The representative immunofluorescence images and its quantified results of TREM2 in the hippocampus of different groups (original magnification, 200×). (**C-D**) The representative Western blotting image and its quantified results of TREM2 in the hippocampus of different groups. (**E**) The ELISA results of sTREM2 level in the hippocampus of different groups. (**F**) The results of hippocampal ADAM17 activity of different groups. (**G**) The RT-PCR results of ADAM17 mRNA in the hippocampus of different groups. Data were presented as means ± SDs.

in the hippocampus of mice. As shown in Figure 5E–G, there was an elevation in pro-inflammatory factors (IL-1β and TNF-α) and a reduction in anti-inflammatory factor (IL-10) in the IH group. However, treatment with TAPI-1 alleviated this hyperinflammatory state. Collectively, our findings suggested that restoring TREM2 expression by inhibiting ADAM17 activity effectively ameliorated neuroinflammation induced by IH.

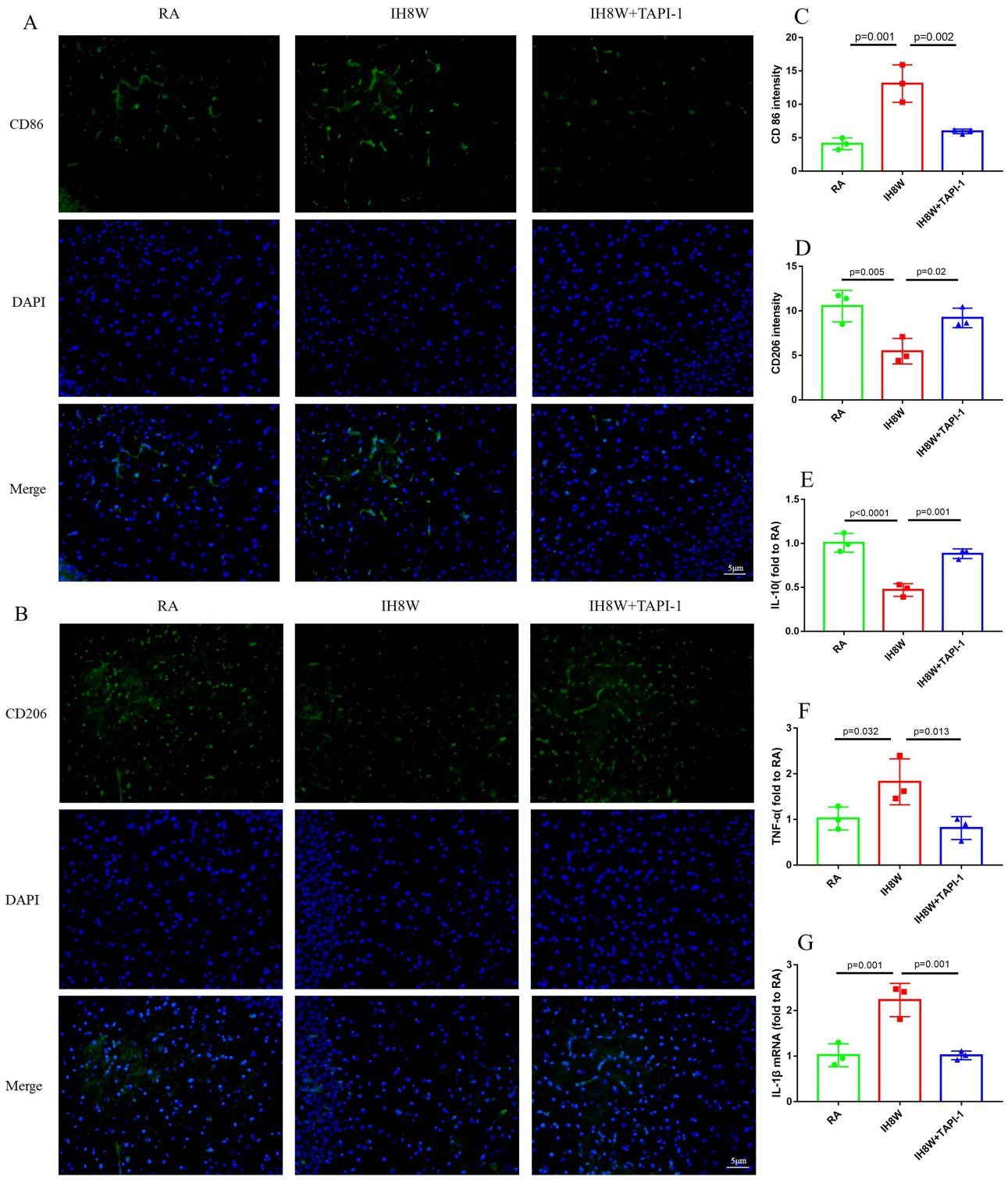

Figure 5 ADAM17 inhibitor alleviated the neuroinflammation in the hippocampus of IH-exposed mice. (**A**–**D**) Representative immunofluorescence images and their quantified results of CD 86 (**A** and **C**) and CD206 (**B** and **D**) in the hippocampus of different groups (original magnification, 200×). (**E**–**G**) The RT-PCR results of IL-10 mRNA (**E**), TNF-α mRNA (**F**), and IL-1β mRNA (**G**) in the hippocampus of different groups. Data were presented as means ± SDs.

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The Inhibition of ADAM17 Activity Mitigated the Cognitive Impairment and Neuronal Injury Induced by IH in Mice

In order to investigate the effect of ADAM17 inhibitor on neuronal damage and cognitive impairment in IH-exposed mice, Nissl staining was conducted to assess the quantity and morphology of Nissl bodies, with the purpose of providing insight into the functional status of neurons. The results depicted in Figure 6A illustrated that hippocampal neurons in the RA group and TAPI-1 group exhibited a well-organized and structured arrangement with abundant uniformly distributed

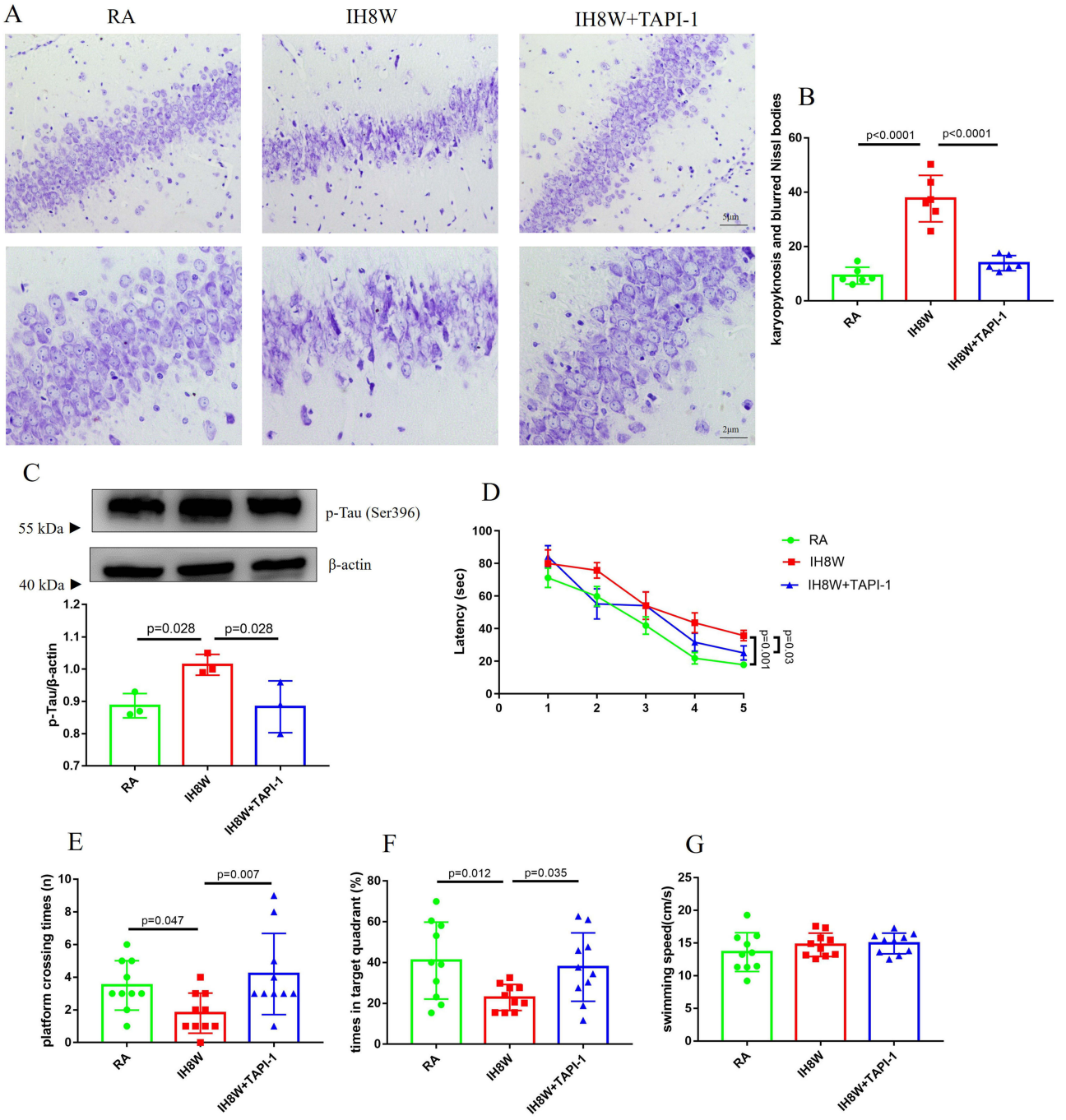

Figure 6 ADAM17 inhibitor mitigated cognitive impairment and neuronal injury induced by IH in mice. (**A** and **B**) The representative Nissl staining images and their quantified results in different groups (original magnification, 200× or 400×). (**C**) The representative Western blotting image and its quantified results of p-Tau in the hippocampus of different groups. (**D**) The escape latency during the training period. (**E**–**G**) The platform crossing times (**E**), times in target quadrant (**F**), and swimming speed (**G**) of mice on day 6. Data were presented as means ± SDs.

Nissl bodies. However, the IH group displayed an irregular arrangement of hippocampal neurons with cellular swelling, blurred nucleoli, and a reduction in the number of Nissl bodies. Statistical analysis indicated an increased proportion of neurons with karyopyknosis and blurred Nissl bodies in mice of the IH group (Figure 6B), which were attenuated by TAPI-1 treatment. Furthermore, increased p-Tau protein within the hippocampus of mice induced by IH was also reversed after TAPI-1 treatment (Figure 6C). In addition, cognitive behavior was assessed in mice by using the Morris water maze test. The IH group showed longer escape latency, lesser number of crossings over the platform, and shorter time in the target quadrant when compared to the RA group. However, these impairments were ameliorated after TAPI-1 treatment (Figure 6D–G and Table S2). These findings suggested that TREM2 recovery by inhibiting ADAM17 activity mitigated IH-induced cognitive impairment and neuronal injury in mice.

Discussion

So far, the management of cognitive impairment caused by IH has become a hot topic because there is no effective medicine. TREM2 is considered as a potential therapeutic target for Alzheimer's disease because of its protective role in neuroinflammation.[25–27] We also observed an increase in sTREM2 levels in OSA patients. Therefore, we performed this study with cell and mouse models to prove our assumption. Our results indicated that TREM2 played a protective role in IH-induced neuroinflammation and neuronal injury by promoting microglia M2 polarization. IH led to the downregulation of full-length TREM2 expression by excessive activation of ADAM17 and then induced neuroinflammation and cognitive impairment. These findings provided convincing evidence of the potential therapeutic strategy (inhibiting ADAM17 activity to reduce TREM2 hydrolysis) for cognitive impairment in OSA.

TREM2 is a crucial protein that regulates neuroinflammatory responses. Zhang et al reported that *Trem2* knockout deteriorated cognitive impairment caused by ischemia in mice, revealing the neuroprotective effect of TREM2.[28] Previous studies indicated that the mice with *Trem2* knockout exhibited an increased p-Tau load in the brain compared to wild-type mice.[29,30] They believed that the lack of TREM2 led to a pro-inflammatory phenotype transformation of microglia, thereby exacerbating neurotoxicity and inducing Tau phosphorylation.[29,30] In this study, we confirmed that IH induced neuroinflammation and increased p-Tau level, which was related to M1 polarization. TREM2 could protect cognitive function and neuronal injury from IH by modulating M1/M2 polarization of microglia.

Some researchers proposed that the downregulation of TREM2 induced by lipopolysaccharide or inflammatory cytokine was potentially mediated through the release of sTREM2 resulting from membrane-bound TREM2 hydrolysis.[31,32] Our previous study observed an elevated level of serum sTREM2 in OSA patients, which was associated with MoCA and nocturnal hypoxemia.[20] In this study, we further confirmed that IH significantly increased sTREM2 levels in the supernatant of BV2 cells. In 2017, Feuerbach et al reported that ADAM17, as the primary protease, was responsible for the release of TREM2 ectodomain through both pharmacological and genetic methodologies.[17] ADAM17 is an ectodomain-shedding enzyme that plays an important role in various diseases by shedding the extracellular domain of membrane-anchored receptors. It has been reported that oxidative stress, an important characteristic of OSA played a role in cognitive impairment, could activate ADAM17 and induce its target receptor shedding.[33,34] Therefore, we conducted further investigations to examine the expression and enzymatic activity of ADAM17. Our results revealed an increase in ADAM17 activity which positively correlated with sTREM2 levels. Chen et al found that in the mouse model of traumatic brain injury, the activity of ADAM17 significantly increased, especially in microglia. ADAM17 inhibitors or gene knockout can promote the polarization of microglia to the M2 type, reduce the release of inflammatory factors such as IL-1β and TNF-α, and improve brain injury.[35] In addition, the study conducted by Schlepckow et al showed that pharmacologically reducing sTREM2 and increasing the expression of TREM2 on microglia could decrease amyloid protein and ameliorate cognitive decline in mice with Alzheimer's disease.[36] Hence, we inhibited the activity of ADAM17, and for the first time, we found that it reduced sTREM2 level and elevated expression of full-length TREM2 in vivo and vitro, thereby promoting microglia M2 polarization and alleviating neuroinflammation and neuronal injury induced by IH. In short, upregulating the expression level of TREM2 by inhibiting ADAM17 activity promoted a neuroprotective effect during IH. However, further investigations of how TREM2 modulated microglia polarization were needed. As well, the level of ADAM17 activity in OSA patients, and whether it could be a biomarker for cognitive impairment, and the long-term effect of ADAM17 inhibition also need further study. In addition, ADAM17 was

overactivated in Alzheimer's disease, whether it caused cognitive impairment by TREM2 shedding, and the effects of ADAM17 inhibition on Alzheimer's disease also need further study.

There were some limitations in our study. First, it has been reported that overexpression of TREM2 can ameliorate cognitive impairment in Alzheimer's disease mice.[13] Based on these findings, this study demonstrated the protective effect of TREM2 on neuroinflammation and neuronal injury induced by IH in BV2 and HT-22 cells instead of TREM2 genetic intervention in IH-exposed mice. However, we observed the neuroprotective role of TREM2 against IH-induced neuronal injury by assessing cognitive impairment-related protein markers in conditioned neuron culture systems, which was confirmed in ADAM17-inhibited mice. Second, the study by Zhong et al reported a pro-inflammatory role of sTREM2, independent of TREM2, in Alzheimer's disease mice.[37] This study mainly focused on investigating the role of TREM2 in IH-induced neuroinflammation and neuronal injury, and the observation of sTREM2 changes was only the product of TREM2 hydrolysis. We did not explore the specific function of sTREM2 itself, further investigations were required. Finally, only male mice were enrolled in this study. Studies have observed distinct sex differences in IH-induced fine motor function impairment and compulsivity but not in IH-induced recollective memory impairments.[38] Although IH could induce microglia activation and neuroinflammation in both genders of mice,[38,39] further studies were also needed to confirm the effect of attenuating neuroinflammation on cognitive impairment mitigation in females.

Conclusions

In conclusion, this study identified that IH caused neuroinflammation and cognitive impairment by excessive proteolysis of TREM2 resulting from ADAM17 overactivation. Inhibition of ADAM17 activity could restore the expression of TREM2 and then protect cognitive function from IH. Given the lack of TREM2-targeted therapies, this study suggests a promising therapeutic strategy for cognitive impairment in patients with OSA.

Data Sharing Statement

All relevant data are available in the figures and supplementary materials. Any additional information required to reanalyze the data reported in this work paper is available from the corresponding author upon reasonable request.

Ethical Statement

All animal study protocol was approved by the Institutional Animal Use Committee of China Medical University (KT20240116), and all the process of animal experiments were in accordance of the ARRIVE guidelines.

Author Contributions

Jiahuan Xu: Methodology, Software, Validation, Formal analysis, Writing-original draft, and Investigation. Hongyu Jin and Xiaomeng Li: Methodology, Investigation, Software, and Writing-review & editing. Zhiping Jiang and Fangqi Meng: Software, Investigation, Formal Analysis and Writing-review & editing. Wei Wang and Wenyang Li: Conceptualization, Methodology, Writing-review & editing and Supervision. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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