

T817MA regulates mitochondrial dynamics via Sirt1 and Arc following subarachnoid hemorrhage

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Abstract

Spontaneous subarachnoid hemorrhage (SAH) is an acute neurologic emergency with poor outcomes, and mitochondrial dysfunction is known as one of the key pathological mechanisms underlying the SAH-induced early brain injury (EBI). 1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl} azetidin-3-ol maleate (T817MA) is a newly synthesized neurotrophic compound that has been demonstrated to exert protective effects against brain injury. Here, we investigated the effect of T817MA in neuronal injury following experimental SAH both in vitro and in vivo. Primary cultured cortical neurons were treated with oxyhemoglobin (OxyHb) to mimic SAH in vitro, and T817MA at concentrations higher than 0.1 μ M reduced OxyHb-induced neuronal injury. T817MA treatment significantly inhibited lipid peroxidation, reduced neuronal apoptosis and attenuated mitochondrial fragmentation. The results of western blot showed that T817MA markedly reduced the expression of mitochondrial fission proteins, fission protein 1 (Fis-1) and dynamin-related GTPase-1 (Drp-1), but prolonged the expression of the postsynaptic protein activity-regulated cytoskeleton-associated protein (Arc). In addition, T817MA significantly increased the expression of sirtuin 1 (Sirt1), which was accompanied by preserved enzymatic of isocitrate dehydrogenase (IDH2) and superoxide dismutase (SOD). Knockdown of Sirt1 and Arc via small interfere RNA (siRNA) transfection partially prevented the T817MA-induced protection in cortical neurons. Furthermore, treatment with T817MA in vivo significantly reduced brain damage and preserved neurological function in rats. The decreased expression of Fis-1 and Drp-1, as well as the increased expression of Arc and Sirt1 were also observed in vivo. Taken together, these data indicate that the neuroprotective agent T817MA protects against SAH-induced brain injury via Sirt1- and Arc-mediated regulation of mitochondrial dynamics.

Introduction

Spontaneous subarachnoid hemorrhage (SAH) is an acute and potentially catastrophic neurologic emergency, which is mainly caused by intracranial aneurysm, arteriovenous malformations or brain trauma. It accounts for 2%-7% of all stroke cases, but the mortality rates are 32% to 67%, with a third of the survivors dependently [1]. The poor outcomes of SAH patients are correlate with early brain injury (EBI) in the first 72 hours following initial bleeding and delayed cerebral ischemia (DCI), which occurs from 3 to 14 days after injury [2]. Although rebleeding may be controlled by surgical clipping or endovascular treatment, no effective drugs had been found to prevent EBI and DCI in clinical settings [3].

1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl} azetidin-3-ol maleate (T817MA) is a newly synthesized neurotrophic compound that was originally screened as a candidate agent for the regeneration of sciatic nerve axons [4]. T817MA was shown to exert neuroprotective effects against amyloid-beta-induced neurotoxicity, as well as sodium nitroprusside (SNP)-induced mitochondrial dysfunction in cultured neurons [4, 5]. In in vivo models, T817MA was demonstrated to prevent MPTP-induced oxidative stress [6], attenuate amyloid-beta-induced place learning deficits [7], and preserve hippocampal neurogenesis and spatial memory function in rats [8]. The potential use of T817MA for the patients with mild to moderate Alzheimer disease (AD) has been extensively investigated by a phase 2 randomized

clinical trial in 2019 [9]. In addition, a previous study showed that T817MA enhanced motor function recovery from internal capsule hemorrhage in nonhuman primates, and the underlying mechanism was associated with CRMP2-mediated facilitation of synaptic AMPA receptors delivery [10]. More recently, we found that T817MA protected against neuronal injury and brain damage following brain trauma both in vivo and in vitro [11]. However, the effects of T817MA on brain damage and neurological function following SAH have not been determined. In the present study, we investigated the effects of T817MA in experimental SAH models, and also elucidated the potential mechanism with focus on mitochondrial dynamics.

Materials And Methods

2.1. Animals

Adult Sprague-Dawley (SD) rats (about 300 g) and pregnant SD rats were obtained from the Animal Experimental Center of Weifang Medical University (Weifang, China). All institutional and national guidelines for the care and use of laboratory animals were followed. All experimental procedures used in this study were approved by the Animal Ethics Committee of Weifang Medical University (No. 2020073).

2.2. Primary culture of cortical neurons

Cortical neurons were primary cultured from embryonic rats using our previously described methods [12]. Briefly, cerebral cortex was dissected and minced in Dulbecco's modified Eagle medium (DMEM) with L-glutamine plus 10% fetal bovine serum at 4°C. Tissues were dissociated by 0.25% trypsin digestion for 15 min at 37°C and gentle trituration. Then, neurons were resuspended in Neurobasal medium (NBM, Invitrogen, Carlsbad, CA, USA) containing 2% B27 supplement (B27, Invitrogen) and seeded in poly-D-lysine (50 µg/mL, 70-150K, Sigma, St. Louis, MO, USA) precoated culture vessels. Neuronal cultures were maintained at 37°C in a humidified incubator (5% CO₂, 95% air, 98% humidity) and the culture medium was changed every other day.

2.3. Experimental SAH models

Primary cultured cortical neurons were treated with 25 µM OxyHb (Ruibio, 07109, German) to mimic neuronal injury following SAH in vitro. The single-hemorrhage model by prechiasmatic cistern injection of autologous blood was used to mimic SAH in vivo. Briefly, animals were anesthetized in the stereotaxic frame, and 0.35 ml un-heparinized fresh arterial blood was injected into the prechiasmatic cistern within 20 s.

2.4. Neuronal toxicity assays

Neuronal toxicity induced by OxyHb treatment was determined by measuring LDH release and calcein signal using kits according to the manufacture's protocols.

2.5. Measurement of lipid peroxidation

Malonyl dialdehyde (MDA) and 4-hydroxynonenal (4-HNE), two index of lipid peroxidation, were determined using assay kits from Cell Bio labs and strictly following the manufacturer's instruction.

2.6. TUNEL staining in neurons

Apoptosis in neurons was determined by measuring DNA fragmentation using a standard TUNEL staining method according to the manufacturer's protocol (Roche, Penzberg, Germany).

2.7. Small interfere RNA (siRNA) transfection

To knockdown the expression of Sirt1 and Arc, Si-Sirt1 (sc-108043), Si-Arc (sc-29721) and control siRNA (Si-Control, sc-37007) were purchased from Santa Cruz (Santa Cruz, CA, USA). These siRNA molecules were transfected using Lipofectamine RNAiMax reagent (Invitrogen) in Opti-MEM medium according to the manufacturer's instructions. After incubation for 48 h, culture media was changed to NBM containing 2% B27 supplement, and neurons were exposed to OxyHb.

2.8. Measurement of brain edema

Brain edema was determined by measuring brain water content using the wet and dry method. After rats were anesthetized and sacrificed by decapitation, the brains were quickly removed and separated through the inter-hemispheric fistula into left and right hemispheres. Tissue samples from injured hemispheres were weighed immediately to get wet weight. After drying in an oven for 48 h at 100°C, the tissues were reweighed to get the dry weight. Brain water content was then calculated using the following formula: % H₂O = (1 - dry weight/wet weight) × 100%.

2.9. Neurological function

Neurological function was evaluated using a 6-point scoring system. For appetite: 0, finished meal; 1, left meal unfinished; 2, scarcely ate. For activity: 0, walk and reach at least three corners of the cage; 1, walk with some stimulation; 2, almost always lying down. For deficits: 0, no deficits; 1, unstable walk; 2, impossible to walk.

2.10. Immunostaining

Brain sections with 4 µm thickness and neurons on the coverslips (fixed with 4% paraformaldehyde) were treated with 0.1% Triton X-100, and then were blocked by 5% bovine serum albumin (BSA). The samples were incubated with the primary antibodies of MAP-2, NeuN, Iba-1, Sirt1 and Arc at 4°C overnight. After being washed by phosphate-buffered saline with Tween-20 (PBST) for three times, the samples were incubated with the secondary antibodies at 37°C for 1 h. Then, 4,6-diamidino-2 - phenylindole (DAPI) was used to stain the nuclei, and the pictures were obtained using a Zeiss fluorescent imaging microscope (Carl Zeiss, Thornwood, NY, USA).

2.11. Western blot

A standard western blot assay was performed using the following primary antibodies: Fis-1 (sc-376447, Santa Cruz), Drp-1 (#8570, Cell Signaling), Sirt1 (#8469, Cell Signaling), Arc (sc-17839, Santa Cruz), and β -actin (ab8226, Abcam). After incubation with secondary antibodies for 1 h, the bands were visualized by using chemiluminescent detection system.

2.12. Statistical analysis

Statistical analysis was performed using SPSS 16.0, a statistical software package. Statistical comparisons were performed using one-way ANOVA. Differences between experimental groups were determined by Student's *t* test. A value of $p < 0.05$ was considered statistically significant.

Results

3.1. T817MA attenuates neurotoxicity induced by OxyHb

To mimic SAH-induced neuronal injury in vitro, cortical neurons were treated with 25 μ M OxyHb. Then, T817MA at different concentrations were added into the culture medium. The results of LDH release assay showed that OxyHb-induced increase in LDH release was significantly decreased by T817MA at 1 and 10 μ M, but not by 0.1 10 μ M T817MA (Fig. 1A). The calcein assay was performed to measure neuronal viability, and the results showed that the OxyHb-induced decrease in calcein signaling was partially prevented by T817MA (Fig. 1B). In addition, we performed MAP-2 staining to detect healthy neurons (Fig. 1C), and the results showed that 1 and 10 μ M effectively preserved neurons in vitro (Fig. 1D). Thus, 1 μ M T817MA was used in following experiments.

3.2. T817MA inhibits oxidative stress and apoptosis

To investigate the effects of T817MA on oxidative, we measured the levels of MDA and 4-HNE, two lipid peroxidation markers. The results showed that OxyHb apparently increased the levels of MDA (Fig. 2A) and 4-HNE (Fig. 2B), both of which were alleviated by T817MA. We also performed TUNEL staining to detect apoptotic cell death in neurons (Fig. 2C), and the results showed that the increased number of TUNEL-positive cells induced by OxyHb was partially nullified by T817MA (Fig. 2D).

3.3. T817MA regulates mitochondrial dynamics in vitro

To investigate the effect of T817MA on mitochondrial dynamics, we detected the morphological changes of mitochondria using mito-tracker staining. As shown in Fig. 3A, healthy neurons exerted tubulated mitochondria, whereas injured neurons showed fragmented mitochondria. OxyHb treatment significantly increased the percentage of fragmented and intermediate mitochondria, which was partially prevented by T817MA (Fig. 3B). Next, we performed western blot to detect the expression of Fis-1 and Drp-1, two mitochondrial dynamic proteins (Fig. 3C). The results showed that the OxyHb-induced increase in Fis-1 and Drp-1 expression were attenuated by T817MA treatment (Fig. 3D).

3.4. T817MA activates Sirt1 and Arc after OxyHb treatment

OxyHb treatment significantly reduce the enzymatic activity of IDH2 in neurons, which was partially abolished by T817MA (Fig. 4A). After transfection with Si-Sirt1, knockdown of Sirt1 reduced the effect of T817MA on IDH2 activity. As shown in Fig. 4B, a similar result on SOD activity was also observed. The results of western blot showed that OxyHb decreased the expression of Sirt1, but T817MA apparently increased Sirt1 expression in neurons (Fig. 4C). In addition, we measured Arc expression at different time points with or without T817MA treatment, and the results showed that OxyHb increased the expression of Arc from 1 to 12 h, which peaked at 6 h (Fig. 4D). Treatment with T817MA further increased Arc expression at 1, 6 and 12 h, and prolonged increase in Arc expression to 24 h after OxyHb treatment (Fig. 4D).

3.5. Role of Sirt1 and Arc in T817MA-induced protection

To confirm the involvement of Sirt1 and Arc in T817MA-induced protection, neurons were transfected with Si-Sirt1 and Si-Arc to knockdown the expression of these proteins. The results of LDH assay (Fig. 5A) and calcein assay (Fig. 5B) showed that the T817MA-induced protection was partially prevented by downregulation of Sirt1 or Arc. As shown in Fig. 5C, a similar result on TUNEL staining was also observed. Also, we performed western blot to detect mitochondrial dynamic proteins after knockdown of Sirt1 and Arc (Fig. 5D), and the results showed that T817MA-induced regulation of Fis-1 and Drp-1 were attenuated by Si-Sirt1 or Si-Arc transfection (Fig. 5E).

3.6. T817MA alleviates brain damage following SAH in vivo

To further investigate the potential protective effects of T817MA in vivo, rats were orally administrated with 30 mg/kg T817MA for 20 days and treated with SAH. We measured brain water content to determine brain edema, and the results showed that the SAH-induced brain edema was markedly reduced by T817MA (Fig. 6A). The results of neurological scores showed that T817MA significantly preserved neurological function after SAH (Fig. 6B). Then, we performed immunostaining using NeuN and Iba-1 antibodies (Fig. 6C), and the results showed that SAH resulted in decrease in NeuN expression and increase in Iba-1 expression. As shown in Fig. 6D, the number of NeuN-positive cells in SAH + T817MA group was higher than that in SAH group, whereas the number of Iba-1-positive cells in SAH + T817MA group was lower than that in SAH group.

3.7. T817MA regulates mitochondrial dynamics and oxidative stress

Next, we measured the levels of MDA and 4-HNE in vivo, and the results showed that the SAH-induced increases in MDA (Fig. 7A) and 4-HNE (Fig. 7B) were both partially reversed by T817MA. The immunostaining using DHE antibody was performed to determine oxidative stress in vivo (Fig. 7C), and the results showed that the SAH-induced increase in DHE fluorescence intensity was partially abolished by T817MA treatment (Fig. 7D). The expression of Fis-1 and Drp-1 were detected by western blot (Fig. 7E), and the results showed that the SAH-induced increases in Fis-1 and Drp-1 expression were both attenuated by T817MA (Fig. 7F).

3.8. T817MA activates Sirt1 and Arc in vivo

We also measured the enzymatic activities of IDH2 (Fig. 8A) and SOD (Fig. 8B) in vivo, and the results showed that the reduced activities of these two enzymes were significantly preserved by T817MA. Immunostaining with Arc antibody was performed to detect Arc expression in brain sections (Fig. 8C), and the results showed that T817MA markedly increased Arc expression following SAH (Fig. 8D). As shown in Fig. 8E, we also detected Sirt1 expression in vivo. SAH resulted in a decrease in Sirt1 fluorescence intensity, which was apparently increased by T817MA treatment (Fig. 8F).

Discussion

In the present study, neuronal injury following SAH in vitro was mimicked by cultured cortical neurons treated with OxyHb, and in vivo SAH model was established by prechiasmatic cistern injection of autologous blood. Using these experimental models, we found that (a) T817MA protects against OxyHb by inhibiting oxidative stress and apoptosis; (b) T817MA attenuates mitochondrial fragmentation and regulates mitochondrial dynamic proteins; (c) T817MA-induced protection depends on Sirt1 and Arc activation; (d) T817MA alleviates brain damage and neurological deficits after SAH; and (e) T817MA regulates mitochondrial dynamic proteins and activates Sirt1 and Arc in vivo.

T817MA was firstly screened as a candidate therapeutic agent for its neurotrophic potency in the central nervous system (CNS). Its neuroprotective effects have been investigated in many neurological disorders, and our present data extended it to experimental SAH models. In neuron/glia cocultures, the neuronal cell death induced by 10 μM A β or 100 μM H₂O₂ could be prevented by treatment with T817MA at 0.1 and 1 μM for 24 h [4]. In congruent, treatment with T817MA at 0.1 and 1 μM for 8 h attenuated the 300 μM SNP-induced neurotoxicity in a dose-dependent manner [5]. However, our results showed that T817MA at 1 and 10 μM could reduce neuronal loss after OxyHb treatment, but 0.1 μM T817MA had no such effects. In our previous study, T817MA at doses higher than 1 μM was effective in scratch-induced neuronal death, and 0.1 μM T817MA was also ineffective [11]. These contradictory results might due to the differences in administration strategies and experimental models.

Mitochondria are dynamic organelles that maintain their normal physiological functions via continuous fission and fusion. Mitochondrial dynamics are closely related to multiple pathophysiological mechanisms of neuronal death following neurological disorders [13]. Mitochondrial fission refers to the division of a single mitochondrion into two smaller mitochondria, during which Drp-1 and Fis-1 play crucial roles [14]. Mitochondrial fission can maintain mitochondria quality through fragmenting broken mitochondria, but excessive mitochondrial fission is detrimental to the normal function of the mitochondrial network. A previous study showed that inhibition of mTOR attenuates early brain injury by reducing excessive mitochondrial fission after SAH [15]. In addition, two neuroprotective agents, docosahexaenoic acid (DHA) and (-)-Epigallocatechin-3-gallate (EGCG), were both found to alleviate the mitochondrial dysfunction and oxidative stress following SAH via regulating mitochondrial dynamics [16, 17]. In the present study, T817MA treatment significantly reduced the levels of MDA and 4-HNE, two

markers of lipid peroxidation, and also inhibited the expression of DHE in vivo, indicating the decreased levels of oxidative stress. The immunostaining results showed that the percentage of fragmented mitochondria in T817MA-treated group is lower than OxyHb group, which is accompanied by decreased expression of Drp-1 and Fis-1. It has been demonstrated that inhibition of Drp-1 via the selective inhibitor Mdivi-1 protects against early brain injury after SAH via attenuating mitochondrial fission and oxidative stress [18]. Our results showed that these two mitochondrial fission proteins were significantly inhibited by T817MA both in vitro and in vivo, indicating that inhibition of mitochondrial fission might be an involved mechanism.

Sirtuins are a family of evolutionarily conserved class III histone deacetylases that play important roles in longevity. The seven mammalian sirtuins (Sirt1-7) are widely expressed in many tissues, and are involved in various cellular processes, such as proliferation, differentiation, oxidative stress and apoptosis. Sirt1 is the most extensively studied Sirtuin that deacetylates many proteins involved in regulator pathways. Previous studies have been demonstrated that Sirt1 exerts protective effects against brain ischemia and traumatic brain injury (TBI) [8, 19, 20]. In the past few decades, the role of Sirt1 in brain damage and neurological function following SAH are gaining widespread attention. A previous study showed that Sirt1 mediates hypoxic preconditioning- and resveratrol-induced protection against neurovascular dysfunction after SAH [21]. Vellimana et al. reported that activation of Sirt1 is an ideal strategy for harnessing endogenous protection against SAH-induced delayed cerebral ischemia [22]. In the present study, we found that the SAH-induced decrease in Sirt1 expression was significantly increased by T817MA both in vitro and in vivo, which were accompanied by the preserved enzymatic activity of IDH2 and SOD. It is well known that Sirt1 could activate SOD and thereby inhibit oxidative stress in various pathological conditions [23-25]. In addition, when Sirt1 expression was block by Si-Sirt1 transfection, the protection induced by T817MA and the regulation of mitochondrial dynamics were partially prevented. In consistent with our present data that T817MA inhibited microglial activation, a recent study showed that Sirt1 promoted M2 microglia polarization by inhibiting nod-like receptor pyrin domain-containing 3 (NLRP3) inflammasome signaling in experimental SAH [26]. Thus, Sirt1 activation and followed attenuation of oxidative stress are involved in T817MA-induced protection in our experimental models.

Activity-regulated cytoskeleton-associated protein (Arc), also known as activity-regulated gene 3.1 (Arg3.1), is a neuronal specific protein coded by the immediate early gene (IEG) *Arc/Arg3.1* [27]. It is a post-synaptic density (PSD) protein that functions in various aspects of long-term synaptic plasticity, such as long-term potentiation (LTP), long-term depression (LTD) and homeostatic scaling. Previous studies have been demonstrated that Arc is a critical regulator of synaptic efficiency at excitatory synapses via modulating the expression and trafficking of ionotropic glutamate receptor AMPARs [28, 29]. The expression of Arc mRNA and protein can be rapidly induced by synaptic activity, and increased expression of Arc was found following neurological disorders [30]. Our present data showed that treatment with OxyHb significantly increased Arc expression from 1 to 24 h, and the expression peaked at 3 h, which was consistent with a previous study showing that glutamate and NMDA resulted in rapid induction of Arc protein in cultured cortical neurons [31]. In the in vivo experimental SAH model, increased expression of Arc in brain section was observed. A similar results has been shown in our previous

study [32]. The functional role of Arc in learning and memory has been extensively studied, and the involvement of Arc in brain injury are getting more and more attention. Our previous study showed that knockdown of Arc aggravated the traumatic neuronal injury via mGluR1-mediated ER stress and necroptosis [33]. In addition, we found that T817MA differently regulated glutamate receptors after brain trauma [11]. Here, our results from western blot showed that the increased expression of Arc following OxyHb treatment was apparently prolonged by T817MA, especially from 6 to 24 h. To further confirm the involvement of Arc in T817MA-induced protection, we silenced Arc expression via Si-Arc transfection, and the results showed that T817MA-induced protection as well as its regulation on Drp-1 and Fis-1 expression were all partially prevented by Arc knockdown. All these data strongly suggested that T817MA protects against SAH-induced brain injury via Arc activation.

In summary, our present study demonstrated that the neuroprotective agent T817MA protects against neuronal injury, brain damage and neurological dysfunction after SAH both in vitro and in vivo. These protective effects were associated with Arc- and Sirt1-mediated regulation of mitochondrial dynamics and oxidative stress. The potential use of T817MA in the treatment of patients with SAH needs to be further investigated.

Declarations

Funding

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Conflict of interest

There is no conflict of interest.

Ethical approval

All institutional and national guidelines for the care and use of laboratory animals were followed. All experimental procedures used in this study were approved by the Animal Ethics Committee of Weifang Medical University (Weifang, China, No. 2020073).

Informed consent

No need.

Author contribution

WBL designed the study. WWC, FQS, and BW performed the experiments. XXT and RPZ analyzed the data. All authors read and approved the manuscript.

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Figures

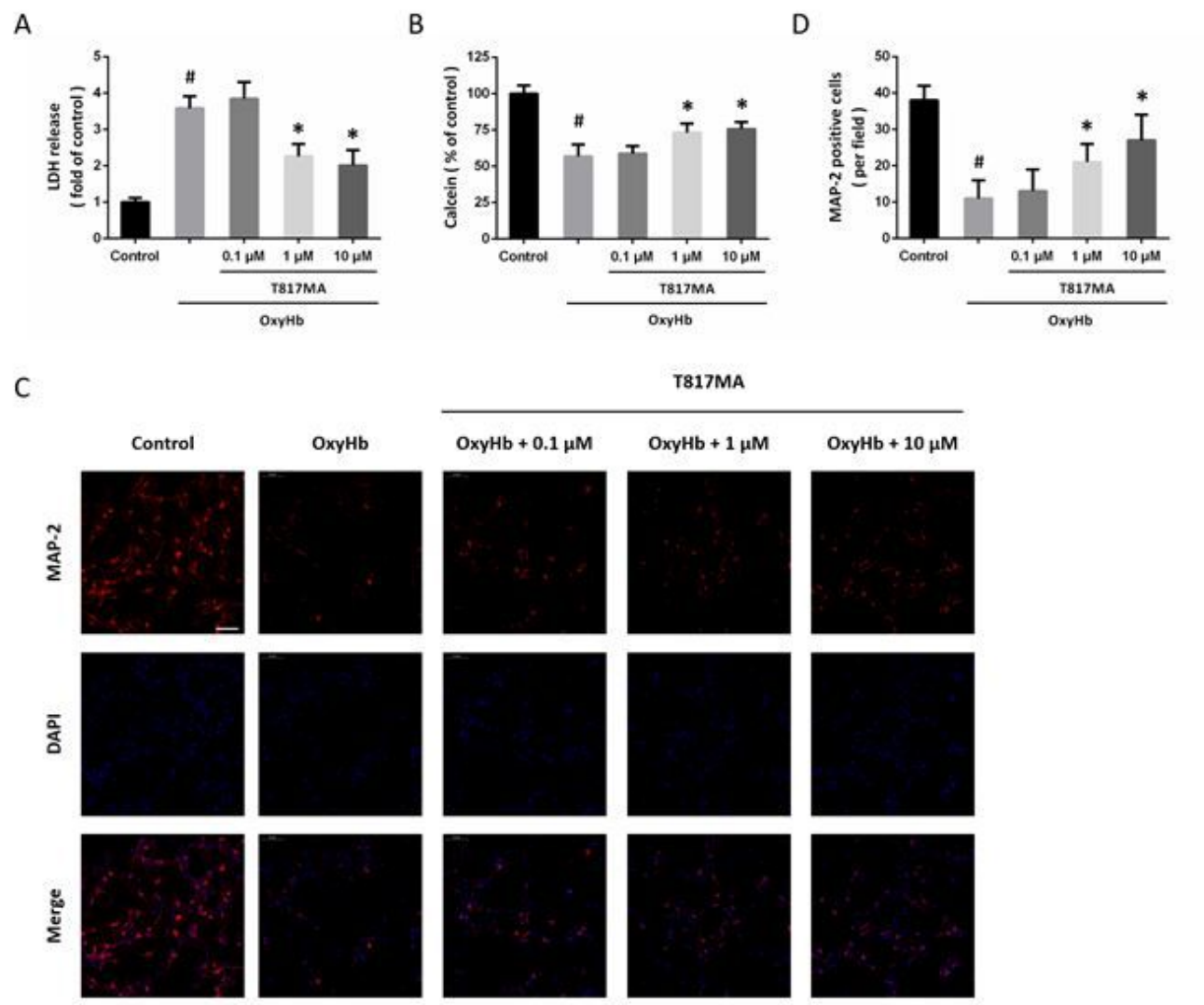


Figure 1

T817MA attenuates neurotoxicity induced by OxyHb. (A) LDH release assay showed that T817MA attenuates OxyHb-induced cytotoxicity. (B) Calcein signal assay showed that T817MA preserves neuronal viability after OxyHb treatment. (C and D) Immunostaining (C) and quantification (D) showed that T817MA protects healthy neurons after OxyHb treatment. Scale bar, 50 μm. Error bars indicate SEM. [#]*p* < 0.05 vs. Control group. ^{*}*p* < 0.05 vs. OxyHb group.

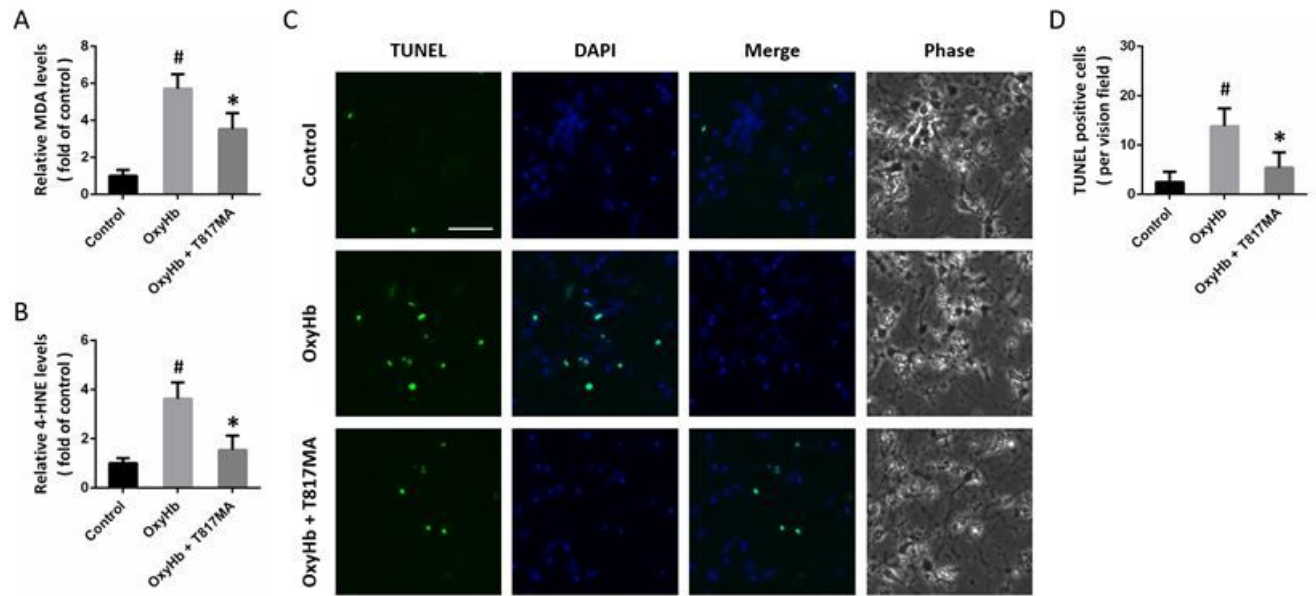


Figure 2

T817MA inhibits oxidative stress and apoptosis. (A and B) Biochemical assays showed that T817MA reduced the levels of MDA (A) and 4-HNE (B) following OxyHb treatment. (C and D) TUNEL staining (C) and quantification (D) showed that T817MA attenuated neuronal apoptosis following OxyHb treatment. Scale bar, 50 μ m. Error bars indicate SEM. [#] $p < 0.05$ vs. Control group. ^{*} $p < 0.05$ vs. OxyHb group.

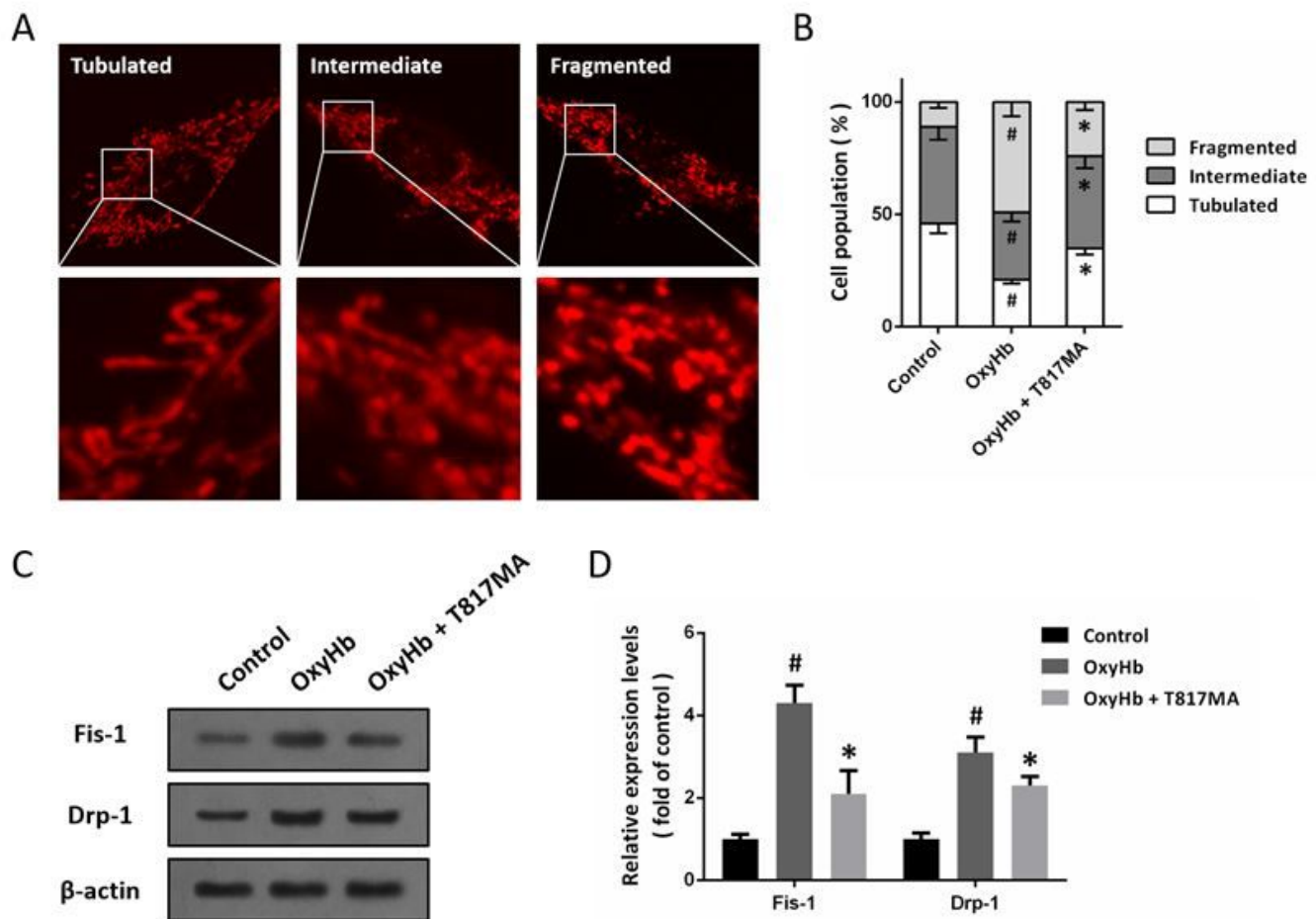


Figure 3

T817MA regulates mitochondrial dynamics in vitro. (A and B) Mito-tracker staining (A) and quantification (B) showed that T817MA alleviated mitochondrial fission following OxyHb treatment. (C and D) Western blot (C) and quantification (D) showed that T817MA inhibited the expression of Fis-1 and Drp-1 after OxyHb treatment. Error bars indicate SEM. [#] $p < 0.05$ vs. Control group. ^{*} $p < 0.05$ vs. OxyHb group.

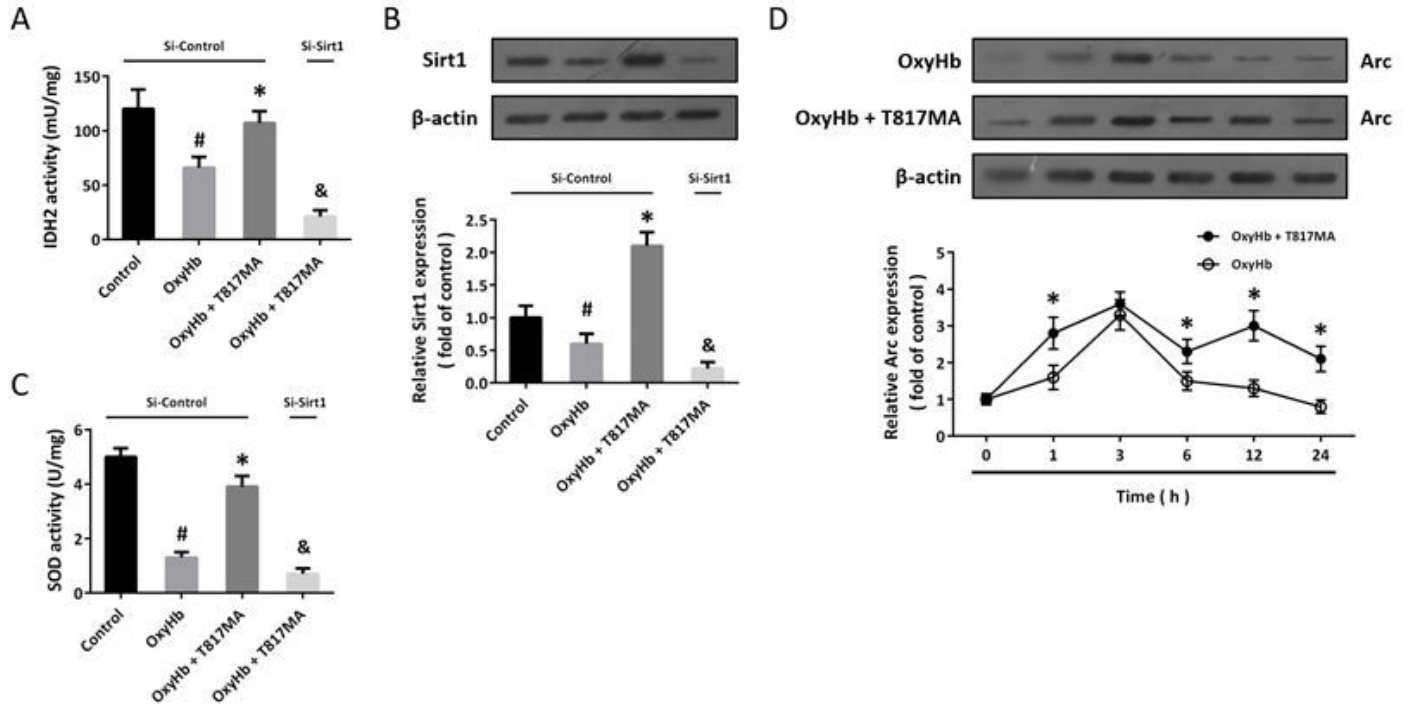


Figure 4

T817MA activates Sirt1 and Arc after OxyHb treatment. (A and B) Biochemical assays showed that T817MA increased the enzymatic activities of IDH2 (A) and SOD (B) following OxyHb treatment. (C) Western blot analysis showed that T817MA increased Sirt1 expression after OxyHb treatment. (D) Western blot analysis showed that T817MA increased and prolonged Arc expression within 24 h after OxyHb treatment. Error bars indicate SEM. [#] $p < 0.05$ vs. Control group. ^{*} $p < 0.05$ vs. OxyHb group. [&] $p < 0.05$ vs. Si-Control group.

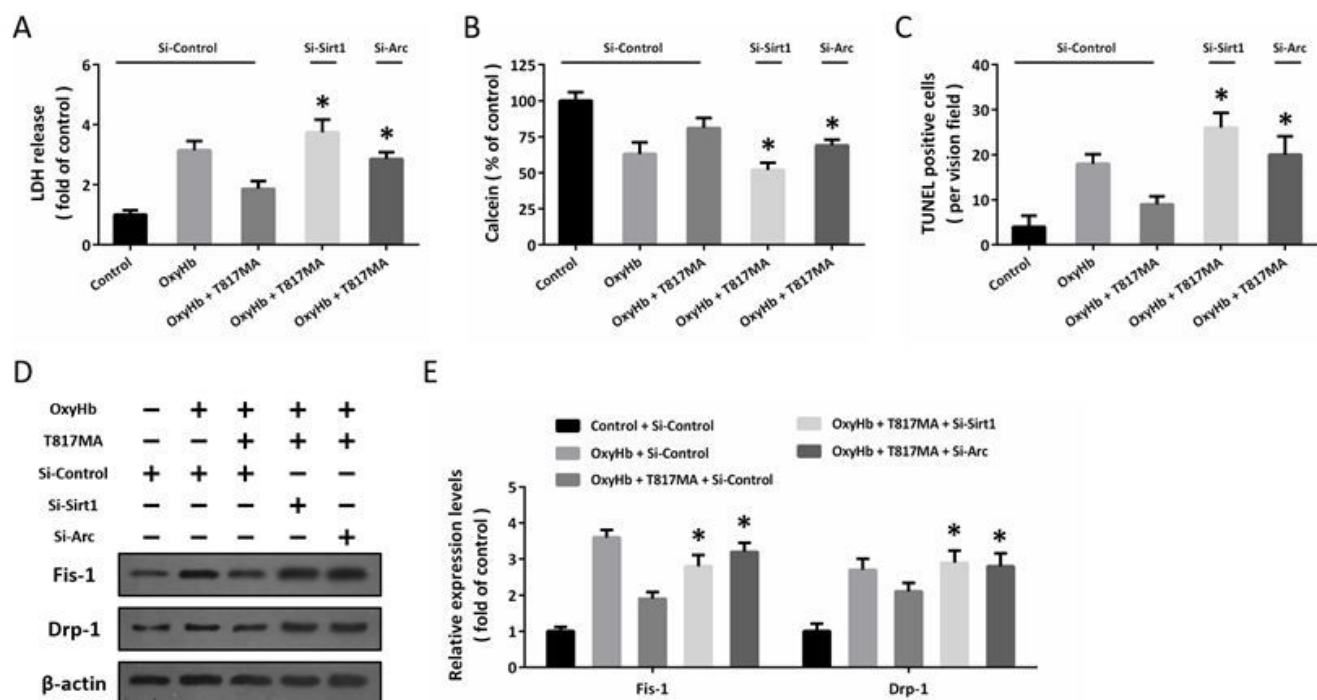


Figure 5

Role of Sirt1 and Arc in T817MA-induced protection. (A) LDH assay showed that T817MA-induced decrease in LDH release after OxyHb treatment was attenuated by Sirt1 or Arc knockdown. (B) Calcein signal assay showed that T817MA-induced increase in neuronal viability after OxyHb treatment was attenuated by Sirt1 or Arc knockdown. (C) TUNEL staining showed that T817MA-induced decrease in neuronal apoptosis after OxyHb treatment was attenuated by Sirt1 or Arc knockdown. (D and E) Western blot (D) and quantification (E) showed that T817MA-induced regulation on Fis-1 and Drp-1 expression after OxyHb treatment was attenuated by Sirt1 or Arc knockdown. Error bars indicate SEM. * $p < 0.05$ vs. Si-Control group.

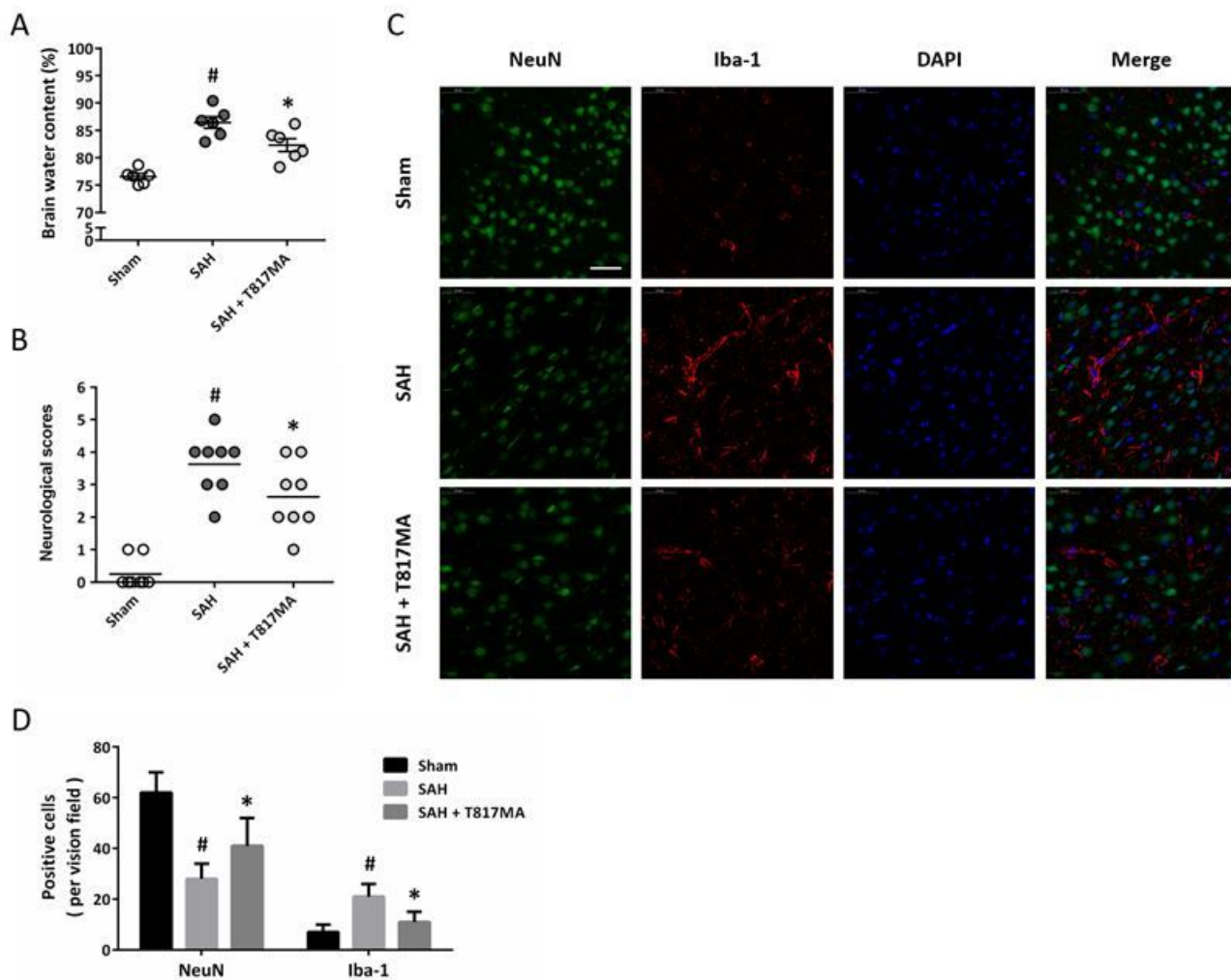


Figure 6

T817MA alleviates brain damage following SAH in vivo. (A) Brain water content assay showed that T817MA inhibited the SAH-induced brain edema. (B) The results of neurological scores showed that T817MA preserved neurological function following SAH. (C and D) Immunostaining (C) and quantification (D) showed that T817MA protected neurons and inhibited microglial activation after SAH. Scale bar, 50 μ m. Error bars indicate SEM. [#] $p < 0.05$ vs. Sham group. ^{*} $p < 0.05$ vs. SAH group.

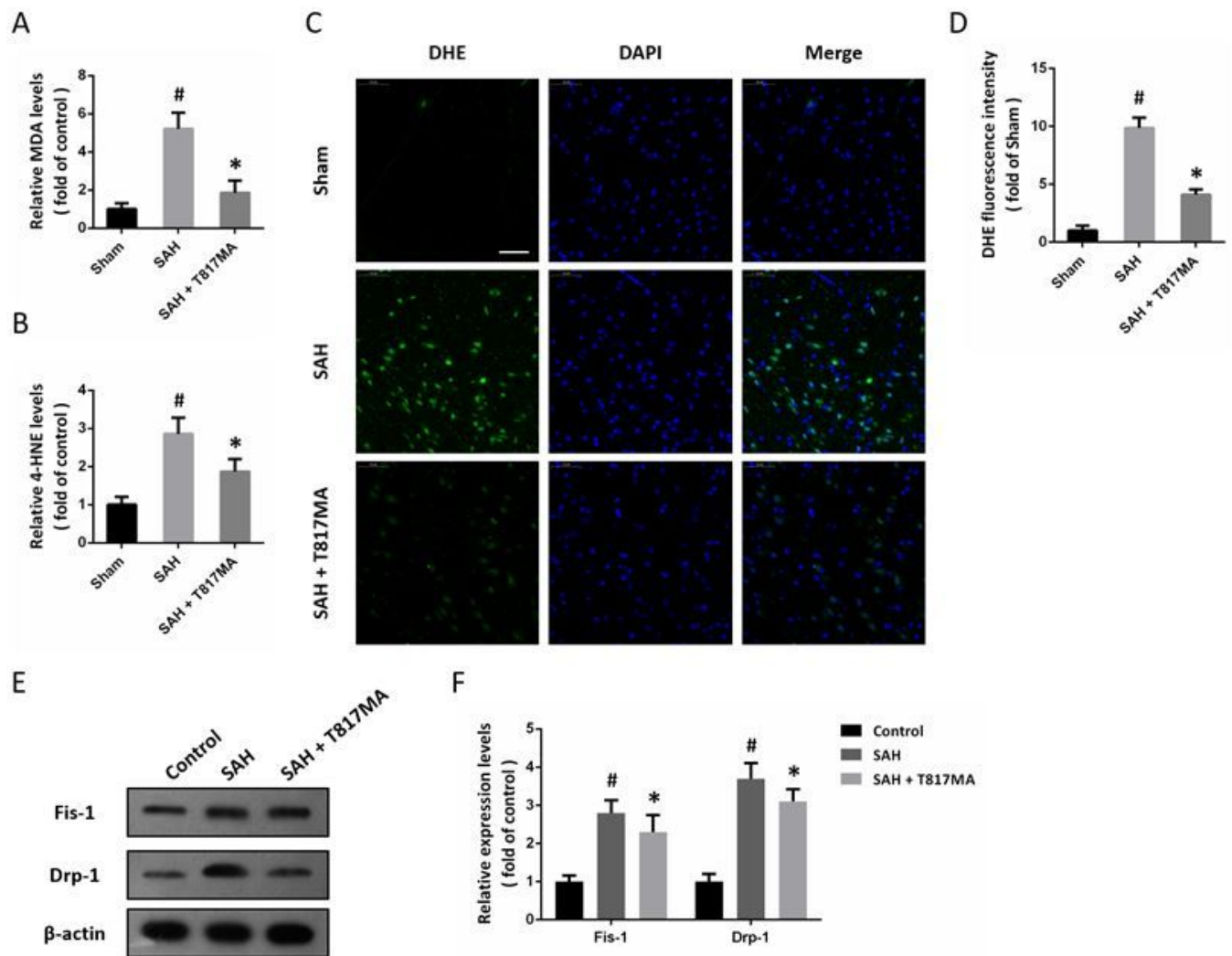


Figure 7

T817MA regulates mitochondrial dynamics and oxidative stress. (A and B) Biochemical assays showed that T817MA reduced the levels of MDA (A) and 4-HNE (B) after SAH. (C and D) DHE staining (C) and quantification (D) showed that T817MA inhibited oxidative stress after SAH. (E and F) Western blot (E) and quantification (F) showed that the SAH-induced expression of Fis-1 and Drp-1 was attenuated by T817MA in vivo. Scale bar, 50 μ m. Error bars indicate SEM. [#] $p < 0.05$ vs. Sham group. ^{*} $p < 0.05$ vs. SAH group.

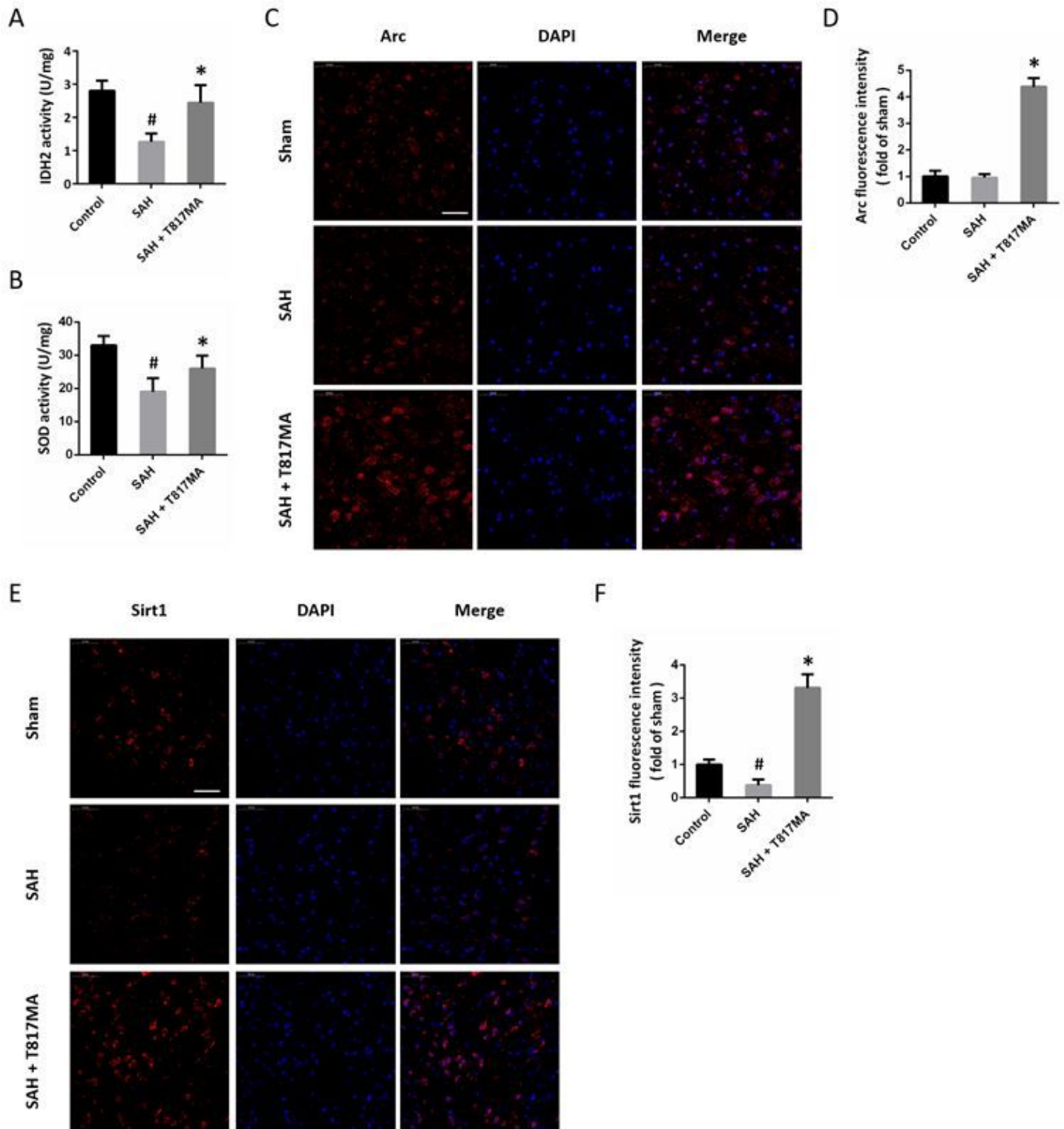


Figure 8

T817MA activates Sirt1 and Arc in vivo. (A and B) Biochemical assays showed that T817MA preserved the enzymatic activities of IDH2 (A) and SOD (B) following SAH. (C and D) Immunostaining (C) and quantification (D) showed that T817MA increased Arc expression following SAH. (E and F) Immunostaining (C) and quantification (D) showed that T817MA increased Sirt1 expression following SAH. Scale bar, 50 μ m. Error bars indicate SEM. [#] $p < 0.05$ vs. Sham group. ^{*} $p < 0.05$ vs. SAH group.