Research Square

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Taurine Promotes Axonal Sprouting Through Mitochondrial Improvement in Stroke

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Abstract

Background

Brain plasticity including axonal sprouting has been recognized in restoring motor function in ischemic stroke. Mitochondrion plays a crucial role in determining axonal sprouting in ischemic injury. Taurine (TAU) could protect brain against experimental stroke as one of the richest amino acids. However, the role of TAU on axonal sprouting and the specific potential mechanism on mitochondria of stroke were unclear.

Methods

Focal cerebral cortical ischemia in C57BL/6 mice was preceded. Motor function was assayed by the Rota-Rod test on D7, D14, and D28 after stroke. Axonal sprouting was detected using immunocytochemistry with biotinylated dextran amine (BDA). The expressions of mitochondrial DNA (mtDNA), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PCG-1a) and Transcription factor A of mitochondria (TFAM) were measured by RT-qPCR.

Results

TAU treatment significantly recovered the motor function of focal cerebral cortical ischemic mice. And TAU promoted axonal sprouting. It was also observed that TAU enhanced mtDNA content, increased the levels of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PCG-1a) and Transcription factor A of mitochondria (TFAM).

Conclusions

Collectively, the data illustrated that TAU exerted a promoting influence on axonal sprouting, through mitochondrial improvement in cerebral ischemic stroke.

Background

Stroke is one of the main causes of disability and death worldwide [1]. In the past decade, brain plasticity including axonal sprouting has opened up a new research direction for the treatment of brain injury, which is widely recognized in the functional recovery of an injured brain [2]. Increasing evidence indicates thatexternal interventions can accelerate repair processes [3]. Therefore, targeting to promote axonal sprouting would be a more promising therapy strategy for ischemic stroke.

Axonal sprouting needs ATP to power fundamental developmental processes [4]. As one of the important organelles, mitochondria produce most of ATP [5]. It has been proposed that mitochondria were involved in brain plasticity after brain injury [6]. The effect of the mitochondrion in determining axonal sprouting has been established by multiple recent studies [7–9]. Therefore, the identification of agents promoting axon regeneration by protecting mitochondriamight be an ideal approach.

Taurine (TAU) stored in mammalian brain tissuesasone of the richest amino acids [10], which has been used in neurological diseases containing traumatic brain injury, Alzheimer's, Parkinson's, and ischemic stroke [11–15]. Previously studied indicated that the role of TAU in neurogenesis has been informed [16, 17]. Emerging research suggest that TAU protects brain from experimental stroke by preserving the mitochondrial function [18]. Nevertheless, whether TAU modulates mitochondrial function during axonal sprouting under stroke has been unclear.

Thus, study intended to assess the role of TAU in axonal sprouting against cerebral ischemic injury, as well as to clarify the function of mitochondria on TAU-induced axonal sprouting.

Methods

The experimental protocols were approved by the Committee of Ethics on Laboratory Animals of Hebei General Hospital, according to the recommendations from the Guide for the Care and Use of LaboratoryAnimals of the National Institute of Health.

Experimental Protocols

The aim of this study was to study the effect of TAU on stroke mice (see Fig. 1). This study divided mice randomly into three groups: Sham, Model, and Model + TAU. In the Model + TAU group, TAU was injected subcutaneously for 7 consecutive days. The Rota-Rod test was carried out at 7, 14, 28 days after the stroke. On D7, we injected biotinylated dextran amine (BDA) into the contralateral hemispheric somatosensory cortex. BDA was used as an anterograde neuronal tracer. On D14, the BDA-labeled axonsdensity was detected using immunocytochemistry. The expressions of mtDNA, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PCG-1a) and Transcription factor A of mitochondria (TFAM) were measured on D14 after stroke.

Focal cerebral cortical ischemia model

Focal cerebral cortical ischemia in C57BL/6 mice was preceded according to previously reported (19]. Briefly, C57BL/6 mice were anesthetized, and ligated the right common carotid artery permanently. Then the mice were exposed the right middle cerebral artery (MCA) with the striatalbranch coagulated. The above operations were all performed except for coagulating the striatalbranch in Sham group.

Rota-Rod test

The Rota-Rod test was performed to assess the motor function and carried out on D7, 14 and 28. On the basis of our previously reported [3], we placed the mice on a rotating Rota-Rod cylinder. The speed of the cylinder accelerated in less than 300 seconds from 4 rpm to 40 RPM. The riding time of the mouse on cylinder was recorded three times. The mean value was used for statistical analysis. Each mouse was trained before the tests, and rested for 15minutes after each test.

Biotinylated Dextran Amine (BDA) injection

After anesthesia, 10% BDA was injected into the mice somatosensorimotor cortex of the contralesional hemisphere (2sites/brain, 1μl/site) by stereotactic device with nano injection pump (0.15 μ L / min). The coordinates of the two injection sites were 1) 2.0 mm posterior to the bregma, 1.5 mm lateral to the midline, and 2) 1.1 mm posterior to the bregma, 1.5 mm lateral to the midline. Based on previously reported, the needle (1μl) was kept after injection in situ for 10 min [20].

Immunocytochemistry(ICC)

The brains of mice were with 4% paraformaldehyde (PFA) fixed. Then cryoprotected it with 30% sucroseand sliced into 20 μm. The three coronal sections including the somatosensory motor cortex and infarct cavity (+ 0.74 mm, − 0.46 mm and − 1.58 mm from the bregma) were selected for ICC. The sections were fixed with 5% normal goat serum. After exposure to avid in horseradish peroxidase (1:1000), the sections were overnight at 4 ℃. Then cultured sections in DyLight 633-conjugated goat anti-horseradish peroxidase (1:500, Jackson ImmunoResearch Labs, USA) for 2h. These images were obtained with an upright fluorescence microscope (Olympus, Japan).

Mitochondrial DNA (mtDNA) quantification

The DNeasy Blood and Tissue kit (Qiagen, ermantown, MD, USA) were used to extract total DNA. Real-time PCR (Rt-PCR) running with the system (Applied Biosystems,USA) in the presence of SYBR Green I was used to detect the mitochondrial DNA (mtDNA) copy number (CWBIO, China). Compared with nuclear DNA (rRNA 18S), the relative copy number of mtDNA was measured. The primers for mtDNA and rRNA 18S were shown below [21]:

mtDNA: sense, 5-$G\mathbb{C}\mathbb{C}AGATATAGCA\top\mathbb{C}C$-3,

anti-sense, 5-$G\top CAT\mathbb{C}TG\top\mathbb{C}TGCT\mathbb{C}$-3.

rRNA 18S: sense, 5-$TAGAGGGAC\forall GTGGCG\top C$-3,

anti-sense, 5-$CGCTGAG\mathbb{C}AGTCAGTGT$-3.

Quantitative Real-Time PCR

Trizol reagent (Invitrogen, USA) was used to extract total RNA and the First-strand cDNA synthesis kit (Fermentas International Inc, Canada) to reverse-transcribe into cDNA. In the presence of SYBR Green I (CWBIO, China), the cDNA was amplified by RT-PCR system (Applied Biosystems company of USA). The $2^{-\triangle\triangle CT}$ method was used to calculate relative PCR products with the mouse glyceraldehyde 3-phosphatedehydrogenase (GAPDH) gene as control gene. The related gene sequences for Quantitative Rt-PCR were shown below [22]:

PCG-1a: Forward,5-$CA\mathbb{C}\forall A\mathbb{C}CACAG\forall\forall CAG$-3,

Reverse,5-$GGGTCAGAGG\forall GAGAT\forall AG\top G$-3,

TFAM: Forward,5- $CA\mathbb{C}CAGATGC\forall\ \forall C\top TCAG$ - 3,

Reverse,5- $CTGCTC\top TATAC\top GCTCACAG$ - 3;

GAPDH: Forward,5- $GACATCATAC\top GGCAGG$ - 3,

Reverse,5- $CTCGTGGAGTCTACTGGT$ - 3.

Statistical analysis

Quantitative data were shown as mean ± SEM (standard error of measurement) andconducted with the SPSS 17.0 software. One-way ANOVA was performed for statistical analysis, and then Turkey's post hoc test or Student t-test was used. $P < 0.05$ were considered statistically significant.

Results

TAU improves recovery of motor function in stroke

To observe the impact of TAU on motor functionof stroke patients, Rota-Rod test was used. Compared with the Sham group, the average riding time of the Model group on the 7th, 14th and 28th day was shorter ($P < 0.05$). Whereas, mice stayed much longerin the TAU-treated groups than the Model groups ($P < 0.05$), indicating that TAU could improve the motor function insulted by ischemic stroke (Fig. 2).

TAU promotes axonal sprouting in ischemic stroke.

Then we explored the potential mechanism of TAU on axonal sprouting in stroke. As shown in Fig. 3, a few BDA-labeled axons could be seen in sham group of the ipsilesional cortex. The Model group axonaldensity of BDA-labeled axons was greater than the Sham group; however, the Model + TAU group got the greatest. Interestingly, the same trend was observed in the contralesional cortex. These results suggest that TAU could promote the growth of axonal branches in ipsilateral cortex as well as the contralateral cortex.

TAU restores mitochondrial-related factors of brain after ischemic stroke

In order to explore the underlying mechanisms, the mitochondria were focused on. Indicators of mitochondrial biogenesis were estimated. MtDNA content was significantly decreased in Model group, whereas increased after TAU treatment. Additional, TAU significantly increased the expressions of PGC-1α, and TFAM after stroke (Fig. 4). The data illustrated that TAU could preserve the mitochondrial-related factors in ischemic stroke.

Discussion

This study elucidates that TAU improves the motor functional recovery and restores neurogenesis in ischemic stroke. This effect is possibly caused by improving the mitochondrial function. Our research provides the novel perspective that TAU promotes axonal sprouting via mitochondrial functional improvement in cerebral ischemic stroke.

It has been reported that recovering motor function is vital for disabled stroke patients [20]. Evidences have shown that brain plasticity including axonal spouting was positively concern with motor functional recovery [23]. Therefore, it is of great significance to explorea novel pharmaceutical for promotingaxonal spouting in stroke. TAU is the main intracellular free-amino acids found in most animal tissues (including brain). TAU can pass throughthe blood-brain barrier and exerts its protection after brain ischemia [18]. Many studies have shown that TAU plays a protective role in stroke [24–27]. A study demonstrates that TAU can be used as an experimental treatment for neuronal damages [28]. Schurr [29] has indicated that TAU pretreatment could restore synaptic function in rathippocampal slice. Additionally, Tau could increase the survival rate of newborn neurons and improve adult neurogenesis [16]. However, it is not clear whether TAU affects axonal sprouting in ischemic stroke. Our results found that TAU revived motor function and promoted axonal germinationafter stroke.

The underlying mechanism to promote Tau-improved axon germination has been further explored. Mitochondria, including axon remodeling, may play a crucial effect in controlling neuroplasticity. Impaired mitochondria may impair neural plasticity after stroke [6]. Recent study has shown that neurological diseases may be related to mitochondrial biogenesis [30]. It has also shown that after cerebral ischemia, biological damage may take a toll on the mitochondrial function in vitro studies [31]. Study has shown that mitochondrial DNA copy number preservation mitigates mitochondrial biogenic damage [32]. Our data showed that the mtDNA loss induced by insult injury was restored by TAU-treatment. In addition, PGC-1α could up-regulate the expression in mitochondrial biology or increase it transcription activity [33]. TFAM has been demonstrated to regulate of copy number of mtDNA [34]. We measured the levels of TFAM and PGC-1α. The results indicated that TAU could restore the levels of TFAM and PGC-1α induced by ischemic injury. Collectively, the data indicated that the TAU-induced axonal spouting following stroke partially mediated through enhanced mitochondrial function.

Conclusion

In conclusion, the above results showed that TAU exerted promoting effect on axonal spouting after stroke through the regulation of mitochondria. These findings highlight that TAU may contribute to the therapeutic intervention of mitochondrial targeted axonal remodeling following ischemic stroke.

Declarations

Acknowledgement

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Availability data and materials

The datasets supporting the conclusions of this study are available from the corresponding author on reasonable request.

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Contributions

HB W and WL H designed and guided the subject. WL H, KH Z and XC T did the experiments, collated and analyzed the data. WL H and KH Z completed the writing and repair of the manuscript. All authors reviewed and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

The experimental protocols were approved by the Committee of Ethics on Laboratory Animals of Hebei General Hospital, according to the recommendations from the Guide for the Care and Use of LaboratoryAnimals of the National Institute of Health.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

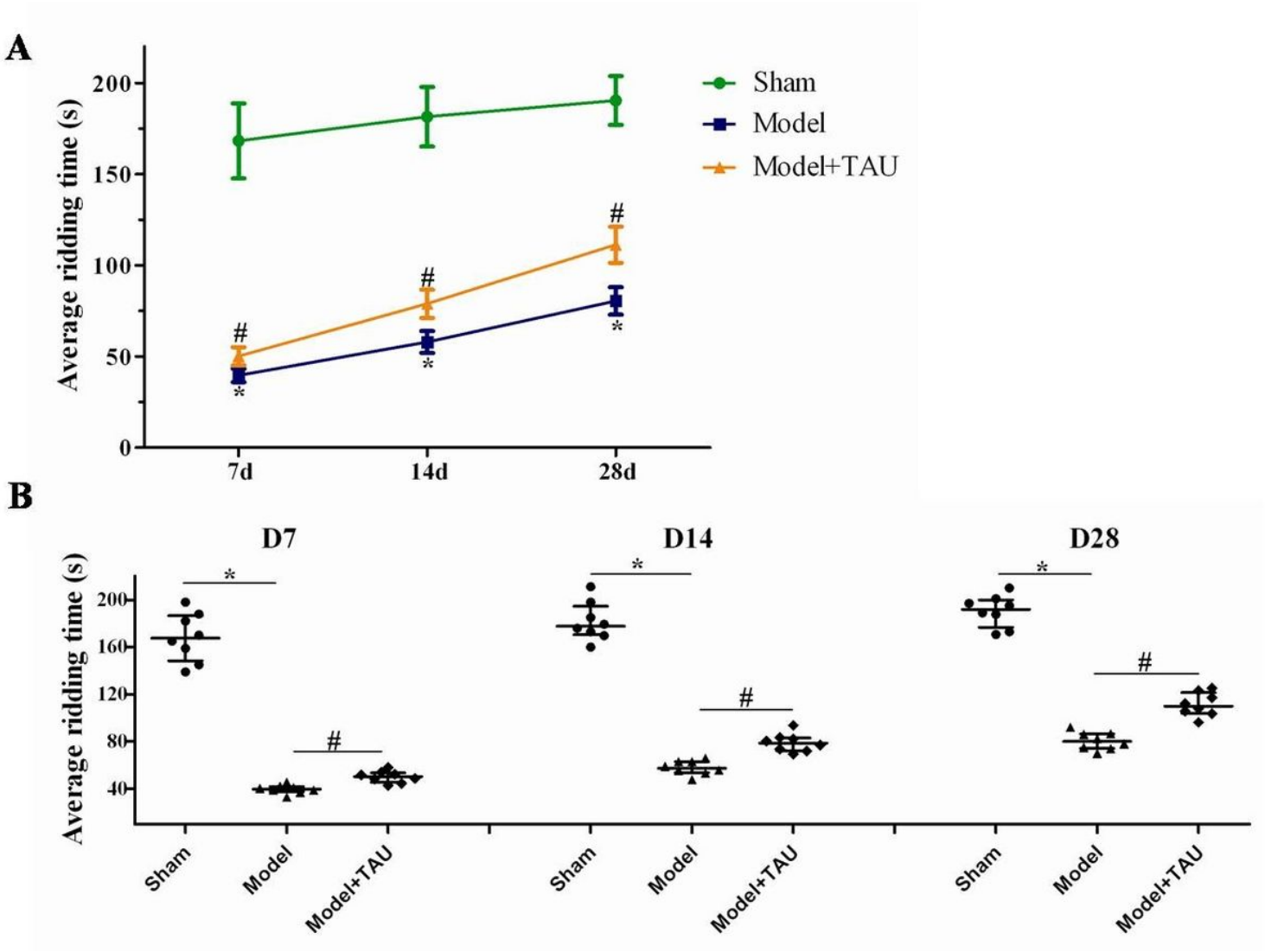

Figure 1

Schematic diagram of experimental design. We divided animals into three groups: Sham, Model, Model+TAU. Mice in Model+TAU groups were injected intraperitoneally with TAU at 30 min after stroke, and during for 7 consecutive day. The Rota-Rod test was carried outat 7, 14, 28 days after the stroke. On

D7, we injected biotinylated dextran amine (BDA) into the contralateral hemispheric somatosensory cortex. After 14 days of tau treatment, the mice were killed by cardiac perfusion. Additionally, mtDNA, the levels of PGC-1α, and TFAM were detected.

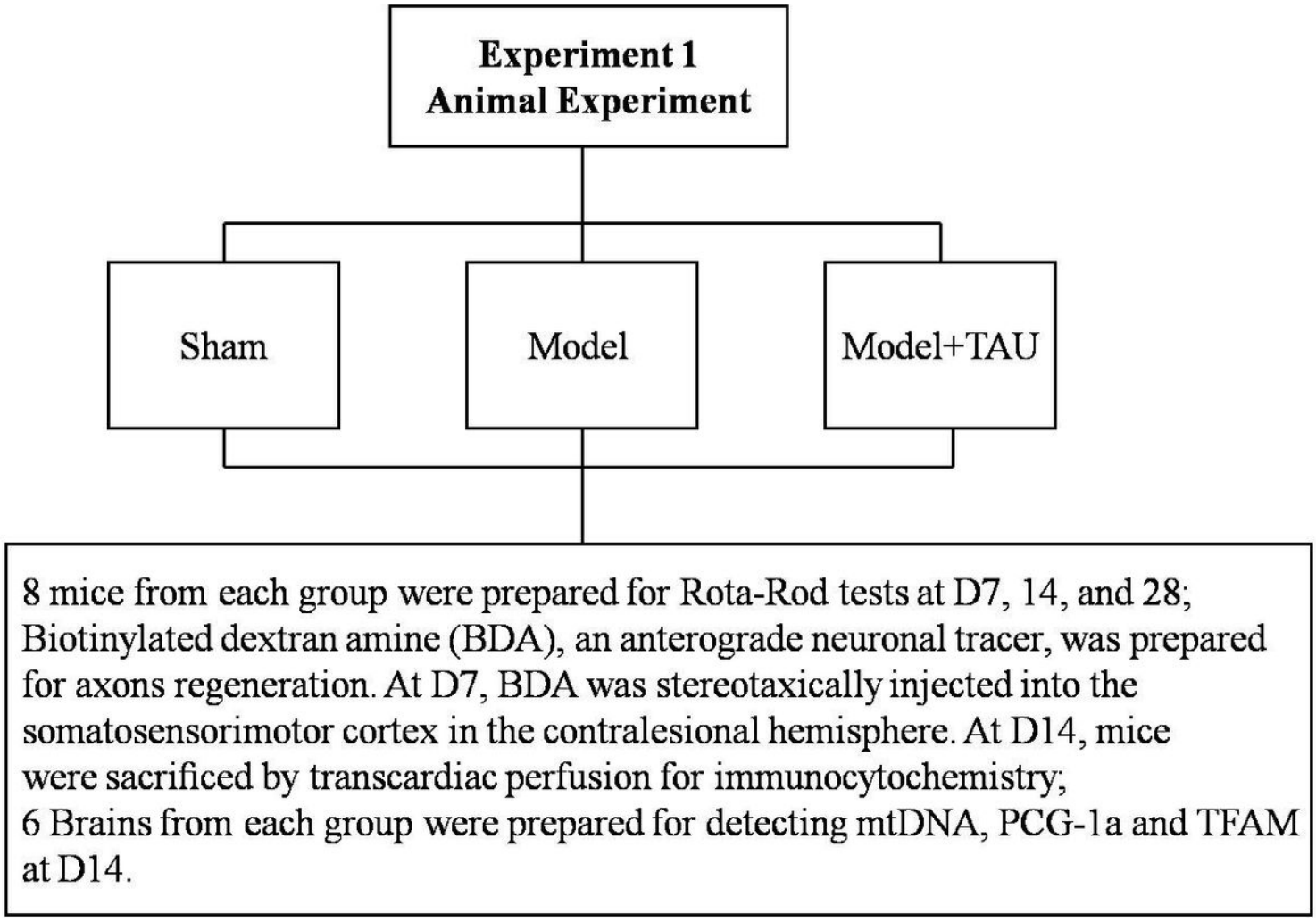

Figure 2

Motional recoveries in the different groups in strokemice. Data showed that TAU remarkably enhanced motional recovery. A and B. Rota-Rod test at D7, D14, and D28. Results were shown as the mean ±S.E.M. n=8. *P<0.05 vs the Sham group, #P <0.05 vs the Model group.

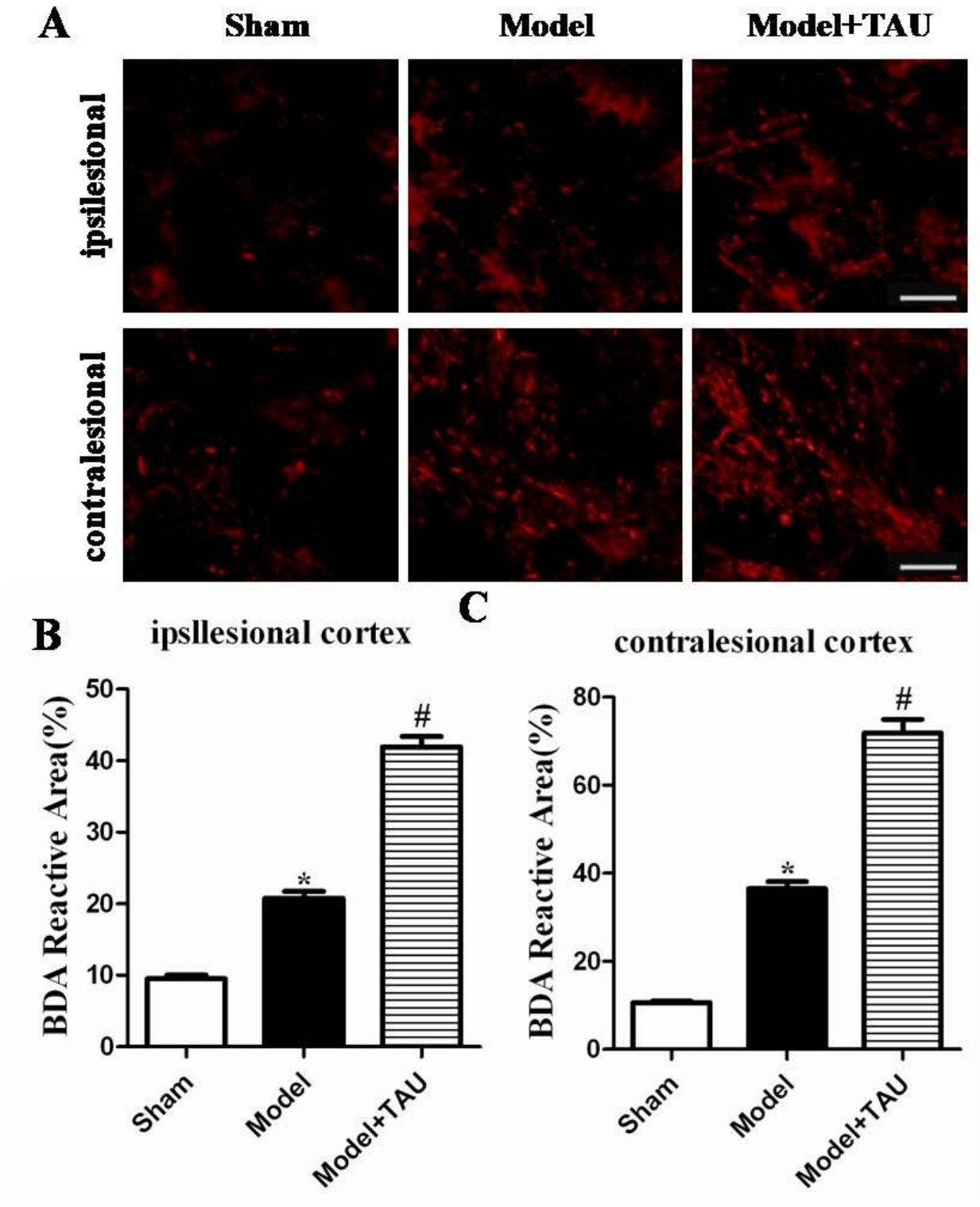

Figure 3

Effect of TAU on axonal sprouting in ischemic stroke. A. Typical images of the BDA-labeled axons in the contralesional cortex but in the ipsilesional cortex, scale=50μm. B. BDA-labeled axon density in ipsilateral cortex. C. BDA-labeled axon density in contralesional cortex. Depicted were the mean ± S.E.M.n=6.*P <0.05 vs the Sham group, #P <0.05 vs the Model group.

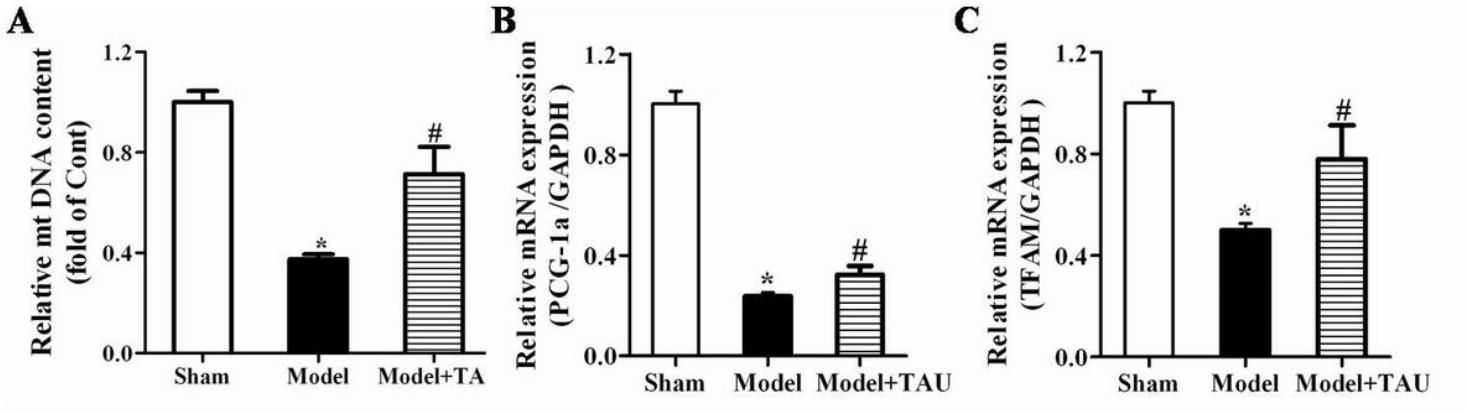

Figure 4

The influence of TAU on mitochondria-related factors in ischemic stroke. A.The content of mitochondrial DNA (mtDNA) in different groups. B. The mRNA expression of PCG-1a detected by RT-qPCR .C. The mRNA expression of TFAM detected by RT-qPCR. Results were showed as the mean ± S.E.M. n=6. *P<0.05 vs Sham group. #P<0.05 vs Model group.