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**Stem Cells from Human Exfoliated Deciduous Teeth Attenuate Trigeminal
Neuralgia in Rats by Inhibiting Endoplasmic Reticulum Stress**

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Abstract

The treatment of trigeminal neuralgia remains a challenging issue. Stem cells from human exfoliated deciduous teeth (SHED) provide optimized therapy for chronic pain. This study aimed to investigate the mechanisms underlying the attenuation of trigeminal neuralgia by SHED. Our findings showed that local transplantation of SHED could relieve trigeminal neuralgia in rats. Further, transmission electron microscopy revealed swelling of endoplasmic reticulum in rats with trigeminal neuralgia. Moreover, SHED inhibited the tunicamycin-induced up-regulated expression of Bip mRNA and protein in vitro. Additionally, SHED decreased the up-regulated expression of Caspase12 mRNA and protein in the trigeminal ganglion of rats caused by trigeminal neuralgia after chronic constriction injury of the infraorbital nerve mode. Our findings demonstrated that SHED could alleviate pain by relieving endoplasmic reticulum stress which provide potential basic evidence for clinical pain treatment.

Keywords Stem Cells from Human Exfoliated Deciduous Teeth; Trigeminal Neuralgia; Endoplasmic Reticulum Stress; Bip-Caspase12

1. Introduction

Trigeminal neuralgia (TN) refers to neuropathic pain originating from the trigeminal nerve. It is among the most common facial pain syndromes and is characterized by recurrent, sporadic, stabbing, paroxysmal, and burning pain in the orofacial region (Bendtsen et al. 2020). Although this neuropathic pain is common, its etiology remains unclear.

Stem cells from human exfoliated deciduous teeth (SHED), which represent a group of immature stem cells, can be easily obtained and isolated using noninvasive procedures (Anoop and Datta 2020). SHED can stay undifferentiated and stable without alterations in their biological and immunological features for a long time, and therefore are important in tissue engineering and stem cell therapy (Fuloria et al. 2020). Under non-neural induction conditions, SHED can express neural markers, including nestin, glutamate decarboxylase, neuron-specific nucleoprotein, glial fibrillary acidic protein, and neurofilament protein, which could be attributed to the dental pulp originating from the neural crest (Fayazi et al. 2021). SHED not only express the early neural marker, nestin, but also later neuron-related enzymes under neural induction culture conditions. SHED have significant nerve regeneration activity and can promote functional recovery after spinal cord injury. Studies have shown that SHED transplantation into a rat model of spinal cord injury significantly improved the recovery of hind limb motor function and inhibited the apoptosis of neurons, astrocytes, and oligodendrocytes; further, it preserved neurofilaments and myelin sheath in the periinjury site (Kunimatsu et al. 2018; Nakamura et al. 2009).

Given the neurogenic differentiation in vivo and neural crest origin of SHED, they provide an alternative model for treating chronic pain, including TN. We previously showed that SHED are crucially involved in attenuating TN; however, the underlying mechanisms remain unclear (Bai et al. 2021b).

Endoplasmic reticulum (ER) stress refers to a disorder of the protein folding process in the ER lumen, which essentially activates the unfolded protein response (UPR). ER stress and UPR are involved in neurodegenerative diseases and are closely associated with chronic pain (Bettigole and Glimcher 2015). Moreover, continuous activation of ER stress may be involved in neuronal injury in response to neuropathic pain stimuli. ER stress associated with neuropathic pain could induce cell apoptosis resulting from neuronal injury through two pathways; namely, the IRE1/ASK1/JNK pathway and the Caspase12 kinase pathway (Zhang et al. 2019). Caspase12 is crucially involved in cell death induced by ER stress, where its expression is increased. Bip, which is also known as GRP78, is the most commonly expressed molecular chaperone protein in early-stage ER stress and is considered as a marker protein of ER stress (Jin et al. 2017). However, the relationship of ER stress with TN and whether SHED can attenuate TN by regulating ER stress remains unclear.

This study aimed to investigate whether SHED could increase the mechanical threshold by inhibiting ER stress via the regulation of Bip and Caspase12. This could provide preclinical evidence supporting the use of dental stem cells in the treatment of TN.

2. Materials and Methods

SHED's Isolation and Culture

Human exfoliated deciduous incisors obtained from children aged 6–8 years were collected as discarded experimental samples in the Hospital of Stomatology, China Medical University. Before obtaining SHED from the samples, we obtained approval from an ethical committee and informed consent from the children's parents. After the pulp was removed from the incisors, it was quickly placed in a precooled medium containing 100 U/mL penicillin/streptomycin double-antibody. Subsequently, the dental pulp was repeatedly rinsed using phosphate buffer solution (HyClone, USA) containing the double-antibody and chopped with a sterile scalpel to form a paste shape (Wu et al. 2021). The minced dental pulp was transferred to a centrifuge tube containing 3 mg/mL type I collagenase (Worthington Biochemical Co, Lakewood, CO, USA) and 4 mg/mL neutral protease (Sigma-Aldrich, St Louis, MO, USA), digested at 37°C for 30 min, and vortexed at 10-min intervals until the tissue was no longer visible. Next, an equal volume of medium was added for neutralization, followed by centrifugation at 1500 rpm for 5 min. The supernatant was then discarded and medium added for resuspension. A single-cell suspension was obtained using a 70- μ m cell sieve (Merck Millipore Ltd, Cork, Ireland) and inoculated in a 10-cm petri dish at a density of 1×10^4 cells/mL. Next, fetal bovine serum (Gibco, USA), L-glutamate (Hyclone), L-ascorbic acid 2-phosphate (Sigma-Aldrich, St. Louis, MO, USA), and penicillin/streptomycin in aMEM (Hyclone, USA) were added and placed in a cell culture incubator (37 °C and 5% CO²). When the cells grow to 80% for passage, they

will be used for subsequent experiments in their third and fifth generations. The cells used in the present study belonged to the same batch used in our previous flow cytometry study, which demonstrated that SHED have the potential for osteogenic and neurogenic differentiation (Bai et al. 2021b).

Co-Culture

In the co-cultivation experiment between SHED and PC12 cells (from Rat adrenal pheochromocytoma), a micropore with a diameter of 0.4 μm was used in the Transwell chamber for establishing an indirect cell co-culture system. During the third to fifth passages, SHED were inoculated to the upper chamber of the 6-well plate at a density of 1×10^5 cells/mL. Moreover, PC12 cells were adjusted to 3×10^5 cells/mL before inoculation to the orifice plate under the chamber. When the cells were completely adherent after 24 h, we added 1 $\mu\text{g/mL}$ tunicamycin (TM, a maturity ER stress model in vitro) to PC12 cells in the lower chamber as the SHED group (SHED co-culture, SHED CC). Further, PC12 cells were cultured alone as the control group. Each group was cultured in three holes and the samples were collected after 12 h for subsequent experiments.

Chronic Constriction Injury of the Infraorbital Nerve Model Establishment and SHED Transplantation

All the experiments met the basic management regulations and ethics of animal experiments of China Medical University (CMU2020192). A rat model of TN was

established through chronic constriction injury of the infraorbital nerve (CCI-ION). All operations were performed under general anesthesia induced by intraperitoneal injection of 2% sodium pentobarbital. The rats were placed in a supine position on the operating table to allow extensive exposure of the oral structure. A surgical incision was made in the mouth to avoid damaging the hair, skin, and tentacles. Nerve ligation was performed using two 4-0 chromic catguts. The surgical incision was closed using 4-0 sutures. In the sham group, the infraorbital nerve was only separated rather than ligated. All operations were aseptically performed without the use of antibiotics (Krzyzanowska et al. 2011).

After being raised for one week, the rats were randomly divided into the CCI-ION and sham groups. Three days after nerve ligation, some of the rats in the CCI-ION group were locally injected once with SHED (5×10^5 cells, 200 μ L, n = 6 rats, CCI-ION+SHED group) at the surgical site, while others were locally injected with serum-free medium (n = 6 rats, CCI-ION + Medium group). Rats in the sham group did not receive injections. After 14 days, the rats were sacrificed through an intraperitoneal injection of 70 mg/kg sodium pentobarbital.

Behavioral Testing

The mechanical threshold was assessed using a calibrated Von Frey filament before and 1, 3, 7, and 14 days after model establishment. The results were assessed using the same boffin by blinded investigators. A suite of calibrated von Frey filaments (weight: 2 g–15 g) was used to apply pressure on the skin above the infraorbital nerve

by bending the filaments. A positive response was indicated by the active withdrawal of the paw and head from the von Frey filaments. The test lasted until the rats showed five positive responses to the same filament. The relevant response frequencies ($[\text{quantity of positive responses}/\text{quantity of total stimuli}] \times 100\%$) were calculated; subsequently, a curve shape of the stimulus-response frequency was plotted. The EF50 value, which is a measure of the mechanical threshold, represents the filament pressure yielding a 50% response frequency based on the plotted curve. An increase in the EF50 value is indicative of relief from mechanical allodynia.

Ultrastructure Analysis through Transmission Electron Microscopy

The rats were sacrificed under deep anesthesia, followed by cutting open of the thoracic cavity, exposing the heart and aorta, carefully inserting a needle into the left ventricle, fixing the needle tip at the beginning of the aorta, and perfusing the right atrium with 0.9% saline solution until the saline from the right atrium becomes clear. Overnight perfusion was performed using 4% paraformaldehyde. Subsequently, the intact trigeminal ganglion (TG) was placed in 4% paraformaldehyde and fixed overnight. Next, a long tissue with a 1-mm³ block was obtained from the end of the TG and soaked in 1% OSO⁴ and uranyl acetate for 1 h and 2 h, respectively. Subsequently, gradient dehydration was performed using ethanol and propylene oxide, followed by tissue embedding with epoxy resin. Ultrathin tissue sections were stained using uranyl acetate. Tissues were observed and photographed using a Hitachi H-7650 transmission electron microscope(Tokyo, Japan).

Real Time-Polymerase Chain Reaction

After the rats were sacrificed under deep anesthesia, 1 cm of the trigeminal nerve was obtained, placed in a refrigerator at -80 °C, and total RNA extracted using the Trizol method. Next, the cells were collected and the culture medium aspirated, followed by washing the cells twice with phosphate-buffered saline. Subsequently, 1 ml Trizol was added to each sample and the cells were thoroughly mixed by pipetting. A fresh 1.5-mL sterile enzyme-free Ep tube was prepared for transferring each sample for RNA reverse transcription. The primer designs were as follows: Caspase12: ATTCCTGGTCTTTATGTCCC, TCATCTGTA TCAGCAGTGGC; Bip: CGGAGGAGGAGGACAAGAAGGAG, ATACGACGGT GTGATGCGGTTG; ACTB: CGTTGACATCCGTAAAGAC, CTGGAAGGTGGA CAGTGAG. The conditions for the real-time polymerase chain reaction were as follows: pre-denaturation at 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 30 s; and finally 95°C for 15 s, 60°C for 30 s, 95°C for 15 s. There were three duplicating holes for each sample and the tests were performed in triplicate.

Western Blot

Initially, denatured total protein was extracted. For protein quantification, based on the number of samples, 50 volumes of A solution plus 1 volume of B solution (50:1) were used to make a BCA working solution (Beyotime, China). After the absorbance value was determined, the protein concentration and absorbance value curves were

plotted to determine the optimal protein concentration. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis, after polymerization of the concentrated gel, pre-stained Marker (Thermo, USA) was added to the sample well, mixed, and transferred to the membrane. The polyvinylidene fluoride membrane was placed into a dish with 5% blocking solution of skimmed milk. After blocking, the membrane was incubated in the dish with primary antibody Caspase12/Bip (Abcam, Britain) overnight at 4°C. On the next day, it was removed, washed with tris-buffered saline Tween 20, and incubated with the secondary antibody (Abcam, Britain). The reaction occurred on a shaker at room temperature for 1–2 h. Finally, luminescence was achieved using the liquid in the ECL kit.

Statistical Analysis

Data are expressed as the mean $\pm$ standard error of the mean. Data regarding the mechanical threshold were analyzed using a two-way repeated-measures analysis of variance (ANOVA). Between- and among-group comparisons were calculated using Student's t-test and one-way ANOVA, respectively. SigmaPlot 14.0 (CA, USA) was used to create the artwork. Statistical significance was set at $p < 0.05$.

3. Results

Local transplantation of SHED reversed hypersensitivity due to CCI-ION

The CCI-ION group showed a significantly decreased mechanical threshold at 1, 3, 7, and 14 days after nerve ligation ($P < 0.01$, for all timepoints). As shown in Fig. 1, the TN model was successfully established; moreover, mechanical allodynia in this model could last for 8 weeks (Bai et al. 2021a). As shown in Fig. 2, after local SHED injection, there was a significant increase in the mechanical threshold at 3, 7, and 14 days ($P < 0.01$, for all time points). Compared with the control group, the SHED group showed a significantly increased mechanical threshold at 3, 7, and 14 days ($P < 0.01$, for all timepoints). This indicates that SHED may reverse the mechanical hypersensitivity and allodynia. Medium administration did not significantly alter the mechanical threshold compared with the baseline level ($P > 0.05$).

Trigeminal nerve ligation caused ER stress in the TG

We examined TG tissues of rats in the CCI-ION group through transmission electron microscopy. This indicated that trigeminal nerve ligation caused ER swelling and abnormal formation as shown in Fig. 3a. The sham group showed no obvious abnormal ER morphology as shown in Fig. 3b.

SHED could reduce TM-induced Bip expression in PC12 cells

Compared with the control group, the TM group showed significantly up-regulated Bip mRNA expression after 24-h TM stimulation ($P = 0.033$ Fig. 4), which suggests that TM could increase Bip expression in PC12 cells. Compared with the TM group, SHED administration significantly inhibited upregulated Bip mRNA expression ($P =$

0.013 Fig. 4). Compared with the control group, the TM group showed significantly upregulated Bip protein expression after 24-h TM stimulation ($p = 0.01$ Fig. 5). Compared with TM group, SHED administration inhibited upregulated Bip protein expression ($p = 0.006$ Fig. 5). These findings suggest that SHED may inhibit TM-induced upregulated Bip expression in PC12 cells.

Effect of local SHED transplantation on Caspase12 levels in the TG

Compared with the sham group, the CCI-ION group showed significantly upregulated Caspase12 mRNA ($p = 0.006$, Fig. 6) and protein ($p = 0.005$, Fig. 7) expression in the TG at 14 days after nerve ligation. This suggests that infraorbital nerve ligation could increase Caspase12 expression in the TG. Compared with the CCI-ION group, the SHED group showed inhibition of the up-regulation of Caspase12 mRNA ($p = 0.001$, Fig. 6) and protein ($p = 0.009$, Fig. 7) expression at 14 days after SHED transplantation. This suggests that local SHED transplantation may inhibit the up-regulation of Caspase12 expression in the TG caused by infraorbital nerve ligation.

4. Discussion

Our findings suggest that SHED could alleviate TN by regulating Bip and Caspase12 expression induced by ER stress, which could provide alternative treatment strategies for TN.

Stem cells, which are self-replicating cells with various differentiation potentials, have been used to treat various neurological diseases, including Parkinson's disease, Alzheimer's disease, schizophrenia, and pain (Shetty and Bates 2016). Direct injection of bone marrow mesenchymal stem cells (BMMSCs) to the lesions of CCI-ION and sciatic nerve CCI rat models was found to increase the mechanical and thermal thresholds (Guo et al. 2011; Abrams et al. 2009). Additionally, intrathecal or intraspinal injection of BMMSCs can alleviate pain (Watanabe et al. 2015). Further, dental stem cells have shown repairing effects in spinal cord injury models (Sakai et al. 2012; Nicola et al. 2016). SHED have better proliferative capabilities than BMMSCs. In our previous study, local SHED transplantation to the region of the injured nerve increased the mechanical threshold of TN, which indicates that SHED could have good antinociceptive effects. Further, our findings demonstrated that SHED transplantation suppresses TN through c-Jun downregulation (Bai et al. 2021a). Additionally, intravenous injection of SHED or conditioned medium from SHED via the left external jugular vein was found to revert mechanical allodynia caused by spinal nerve transection (Hayashi et al. 2021). SHED administration can also relieve diabetic neuropathic pain in diabetic Goto-Kakizaki rats and significantly improve limb function (Xie et al. 2019). In nude mice, intraperitoneally injected SHED can be distributed to various tissues and organs, including the liver, spleen, and kidney (Huang et al. 2009). SHED can not only differentiate into teeth, bone, cartilage, fat, among others, they can also nerve cells under certain induction conditions. SHED originate from the neural crest, which can differentiate into neural tissue and can,

therefore, be used to treat neuropathic diseases. Striatal SHED transplantation in a Parkinson rat model leads to detectable active cells in the brain, followed by alleviation of Parkinson's symptoms (Wang et al. 2010). The neurological regenerative activity of SHED involves inhibiting neuronal apoptosis and promoting axon regeneration (Sakai et al. 2012). Further, lost cells are replaced by differentiated gliocytes under extreme conditions. However, this is the first study to investigate whether SHED could alleviate TN. We successfully established the TN model by ligating the infraorbital nerve in rats, which significantly reduced the mechanical threshold. Local SHED transplantation reversed this hypersensitivity, indicating that SHED could relieve TN symptoms.

The ER is a highly dynamic organelle that is crucial in coordinating signal transduction pathways to ensure cell adaptation, resilience, and survival. ER stress is caused by the accumulation of unfolded or misfolded proteins and the resulting changes in the internal environment could cause imbalanced ER function, which induces UPR (Wu et al. 2016). UPR requires mediation by three cardinal ER stress receptor proteins; namely, inositol requiring enzyme 1, activating transcription factor 6 (ATF-6), and pancreatic ER kinase-like ER kinase. Intrathecal injection of the siRNA of ATF-6 can attenuate neuropathic pain, which demonstrates the crucial role of ER stress in inducing neuropathic pain (Zhan et al. 2015). ER stress in the dorsal root ganglion contributes to the development of pain hypersensitivity after nerve injury (Yamaguchi et al. 2018). Moreover, ER stress in the peripheral nervous system is a significant driver of neuropathic pain (Inceoglu et al. 2015). ER stress

significantly contributes to pain hypersensitivity. The role of ER stress in neuropathic pain has been demonstrated in a rat model of orofacial inflammatory pain. Specifically, injection of complete Freund's Adjuvant was found to attenuate ER stress in the TG by increasing heat hyperalgesia, which suggests that induction of ER stress in the primary sensory neurons could be involved in orofacial pain. ER stress and UPR have been examined in a rat model of neuropathic pain induced by L5 spinal nerve ligation; additionally, nerve ligation contributes to activation of Bip, followed by the IRE-XBP1 and ATF6 pathways. In our study, transmission electron microscopy revealed obvious ER swelling in the TG of rats in the CCI-ION group. Moreover, rats in the CCI-ION group showed increased Caspase12 mRNA and protein expression in the TG, which indicates the involvement of ER stress in TN.

Increased Bip expression in the dorsal horn leads to the induction of ER stress in response to neuronal injury. Our in vitro study revealed that TM stimulation significantly upregulated Bip mRNA and protein expression in PC12 cells; moreover, SHED inhibited upregulated Bip mRNA and protein expression. Caspase12 precursor (procaspase12), which is located on the side of the ER close to the cytoplasm, cleaves and specifically activates Caspase12 during ER stress. Caspase12 was the first identified member of the Caspase family and is strongly correlated with the ER (Van Opdenbosch and Lamkanfi 2019). During cell apoptosis induced by ER stress, there is activation of Caspase12 present in the ER. Cytoplasmic Caspase3 is then activated, which finally triggers cell apoptosis (Jia et al. 2019). There is increased Caspase12 expression only during the occurrence of ER stress. In our in vivo study, Caspase12

mRNA and protein expression were significantly upregulated after nerve ligation; additionally, local SHED transplantation reverted the TN hypersensitivity by effectively downregulating Caspase12 mRNA and protein expression. Taken together, our findings demonstrate that SHED could inhibit ER stress by downregulating Bip and Caspase12 expression, providing novel information for the treatment of trigeminal neuralgia.

5. Conclusions

Our findings demonstrate that SHED can reverse hypersensitivity by inhibiting ER stress via downregulating Bip and Caspase12 expression. These results provide preclinical evidence supporting the use of dental stem cells in TN treatment.

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Author Contributions: Xiaofeng Bai and Xia Zhang contributed to the study conception and design, data acquisition, and manuscript drafting and revision. Zhijie Yang contributed to manuscript drafting, study design, and establishment of the co-culture and pain models. Chun Wang contributed to the collection of behavioral data as well as data acquisition and analysis. Jing Li and Ziqi Zhang contributed to

behavioral data collection; Zhao Tan and Ke Xiao contributed to SHED cultures; Junyi Wang and Junyang Zhang contributed to analysis. All authors gave final approval of the manuscript.

Data Availability Statement: The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Availability of Data and Materials: The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Ethics Approval: All animal experiments were conducted to meet the basic management regulations. Animal experiments were approved by the Research and Ethics Committee of China Medical University (CMU2020192), Shenyang, China.

Consent to Participate: We obtained informed consent from the children's parents before obtaining SHED from exfoliated deciduous incisors.

Competing interests: The authors declare that they have no competing interests.

Consent for Publication: The authors all agree to publish this article.

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Figure Legends

Fig. 1 Changes in the mechanical threshold in the CCI-ION and sham groups; * $P < 0.05$. Compared with the sham group, the CCI-ION group showed an increased mechanical threshold at 1, 3, 7, and 14 days after nerve ligation; [#] $P < 0.05$. Compared with the baseline level, the CCI-ION group showed an increased mechanical threshold at 1, 3, 7, and 14 days after nerve ligation.

Fig. 2 Changes in the mechanical threshold in the sham, medium, and SHED groups; [#] $P < 0.05$: Compared with the baseline level, the SHED group showed increased mechanical threshold at 3, 7, and 14 days after nerve ligation; * $P < 0.05$. Compared with the medium group, the SHED group showed an increased mechanical threshold at 3, 7, and 14 days after nerve ligation.

Fig. 3 Change of the shape of the endoplasmic reticulum after nerve ligation. (a) The arrow shows the normal form of the endoplasmic reticulum. (b) The star shows the abnormal form of the endoplasmic reticulum. Scale bar = 1 μ m, N: Nucleus

Fig. 4 Relative expression level of Bip mRNA in PC12 cells. * $P < 0.05$: the TM group compared with the control group; # $P < 0.05$: the SHED group compared with the TM group.

Fig. 5 Relative expression level of Bip protein in PC12 cells. * $P < 0.05$: the TM group compared with the control group; # $P < 0.05$: the SHED group compared with the TM group.

Fig. 6 Relative expression level of Caspase12 mRNA in the TG. * $P < 0.05$: the CCI-ION group compared with the sham group; # $P < 0.05$: the SHED group compared with the CCI-ION group (n = 6 rats in each group).

Fig. 7 Relative expression level of Caspase12 protein in the TG. * $P < 0.05$: the CCI-ION group compared with the sham group; # $P < 0.05$: the SHED group compared with the CCI-ION group (n = 6 rats each group).

Figures

Figure 1

Changes in the mechanical threshold in the CCI-ION and sham groups; * $P < 0.05$. Compared with the sham group, the CCI-ION group showed an increased mechanical threshold at 1, 3, 7, and 14 days after nerve ligation; # $P < 0.05$. Compared with the baseline level, the CCI-ION group showed an increased mechanical threshold at 1, 3, 7, and 14 days after nerve ligation.

Figure 2

Changes in the mechanical threshold in the sham, medium, and SHED groups; # $P < 0.05$: Compared with the baseline level, the SHED group showed increased mechanical threshold at 3, 7, and 14 days after nerve ligation; * $P < 0.05$. Compared with the medium group, the SHED group showed an increased mechanical threshold at 3, 7, and 14 days after nerve ligation.

Figure 3

Change of the shape of the endoplasmic reticulum after nerve ligation. (a) The arrow shows the normal form of the endoplasmic reticulum. (b) The star shows the abnormal form of the endoplasmic reticulum. Scale bar = 1 μ m, N: Nucleus

Figure 4

Relative expression level of Bip mRNA in PC12 cells. * $P < 0.05$: the TM group compared with the control group; # $P < 0.05$: the SHED group compared with the TM group.

Figure 5

Relative expression level of Bip protein in PC12 cells. * $P < 0.05$: the TM group compared with the control group; # $P < 0.05$: the SHED group compared with the TM group.

Figure 6

Relative expression level of Caspase12 mRNA in the TG. * $P < 0.05$: the CCI-ION group compared with the sham group; # $P < 0.05$: the SHED group compared with the CCI-ION group ($n = 6$ rats in each group).

Figure 7

Relative expression level of Caspase12 protein in the TG. * $P < 0.05$: the CCI-ION group compared with the sham group; # $P < 0.05$: the SHED group compared with the CCI-ION group ($n = 6$ rats each group).