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Sigma-1 receptor protects against endoplasmic reticulum stress-mediated apoptosis in mice with cerebral ischemia/reperfusion injury

Xuemei Zhao[1,2] · Lin Zhu[1] · Danyang Liu[1] · Tianyan Chi[1] · Xuefei Ji[1] · Peng Liu[1] · Xuexue Yang[1] · Xinxin Tian[1] · Libo Zou[1]

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Abstract

Reports have showed that Sigma-1 receptor (Sig-1R) activation can protect neurons against cerebral ischemia/reperfusion (I/R) injury in mice and alleviate endoplasmic reticulum (ER) stress in cultured cells, but little known is about the protective role of Sig-1R on ER stress induced by cerebral I/R. The purpose of this study was to determine whether Sig-1R exerts a protective effect against ER stress-mediated apoptosis in cerebral I/R using a 15-min bilateral common carotid artery occlusion (BCCAO) mouse model. At 72 h after reperfusion in BCCAO mice, we found that Sig-1R knockout (Sig-1R KO) significantly increased terminal dUTP nick-end labeling (TUNEL)-positive cells and nuclear structural damage in cortical neurons. Treatment with the Sig-1R agonist PRE084 once daily for three consecutive days reduced the number of TUNEL-positive cells and improved the ultrastructural damage of neurons in the cerebral cortex. These protective effects could be blocked by the Sig-1R antagonist BD1047. Then, we used BCCAO mice at 24 h after reperfusion to detect the expression of ER stress-mediated apoptotic pathway proteins. We found that expression of the pro-apoptotic proteins p-PERK, p-eIF2α, ATF, CHOP, p-IRE, p-JNK, Bim, PUMA, cleaved-caspase-12 and cleaved-caspase-3 was significantly increased and that expression of the anti-apoptotic protein Bcl-2 was significantly decreased in Sig-1R KO-BCCAO mice compared with BCCAO mice. Meanwhile, we found that treatment with PRE084 twice a day decreased pro-apoptotic protein expression and increased anti-apoptotic protein expression. The effects of PRE084 were blocked by the Sig-1R antagonist BD1047. These results suggest that Sig-1R activation inhibits ER stress-mediated apoptosis in BCCAO mice, indicating that Sig-1R may be a therapeutic target for neuroprotection particularly relevant to ER stress-induced apoptosis after cerebral I/R injury.

Keywords Sigma-1 receptor · Ischemia/reperfusion · ER stress · Apoptosis · Neuron

✉ Libo Zou
libozou@163.com

Xuemei Zhao
84458728@qq.com

Lin Zhu
zhulindedream@163.com

Danyang Liu
1013780534@qq.com

Tianyan Chi
chitianyan@163.com

Xuefei Ji
107953319@qq.com

Peng Liu
807554912@qq.com

Xuexue Yang
1342076292@163.com

Xinxin Tian
157021767@163.com

[1] Department of Pharmacology, Life Science and Biopharmaceutics School, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, Shenyang 110016, Liaoning, People's Republic of China

[2] Department of Pharmacology, Qiqihar Medical University, 333 BuKui Street, JianHua District, Qiqihar 161006, People's Republic of China

Introduction

The endoplasmic reticulum (ER), a site of protein synthesis and folding, can be perturbed by a variety of stresses, including ischemia/reperfusion (I/R) injury [1]. ER stress initiates a cellular self-protective strategy that triggers an unfolded protein response (UPR) to restore normal ER function; ER stress beyond the self-defense capacity can activate apoptotic signals to protect the organism [2]. A recent study has shown that ER stress participates as a contributing factor in several pathophysiological conditions, such as by linking ischemia and apoptosis, to affect numerous cellular processes through the UPR [3]. Conditions of prolonged and uncontrolled ER stress mediating apoptosis and resulting from a dysfunctional UPR can induce the activation of ER transmembrane receptors, such as PKR-like ER kinase (PERK) and inositol-requiring enzyme-1α (IRE1) [4, 5]. Reports have suggested that the UPR signaling switches from pro-survival (adaptive response) to pro-apoptosis (maladaptive response) through activation of the transcriptional induction of the C/EBP homologous protein (CHOP) pathway by PERK receptors and the c-Jun N-terminal kinase (JNK) and caspase-12-dependent pathways by IRE1 receptors to damage neuroprotection [6].

As a molecular chaperone protein, the Sigma-1 receptor (Sig-1R) is mainly expressed on the ER membrane, forming focal contacts between the ER and mitochondria and regulating ER stress to contribute to neuroprotection [7]. Under normal conditions, Sig-1R forms a complex with another molecular chaperone, GRP78/BiP, on the ER membrane. Under conditions of ER stress, Sig-1R dissociates from BiP to interact with inositol 1,4,5-triphosphate receptors (IP3Rs) and stabilizes the IP3R structure [8]. Several functions of the nervous system have been attributed to Sig-1R, including the regulation of neurogenesis, the activity of ion channels, e.g., Ca^{2+} and K^{+} channels, the release of neurotransmitters, such as dopamine, and drug addiction [9]. Sig-1R activation has been shown to protect memory loss in amnesia and aging-related models [10, 11]. Moreover, other observations in both in vivo and in vitro models of ischemia suggest that Sig-1R exerts a neuroprotective effect [12, 13]. As a Sig-1R agonist, DHEA could prevent ischemia-induced neuronal death by 3–48 h after ischemia [14]. However, the neuroprotective mechanism of Sig-1R in cerebral I/R injury-mediated ER stress is still unclear.

Therefore, in this study, we investigated whether Sig-1R protected neurons through ER stress- mediated CHOP, JNK and caspase-12 signaling in the pathophysiology of cerebral I/R injury. Herein, we addressed the neuroprotective roles and the mechanisms of Sig-1R in ER stress-mediated apoptosis in mice subjected to cerebral I/R injury.

Materials and methods

Materials

BD1047 and PRE084 were both purchased from Tocris Bioscience (Britain), and they were dissolved in saline to a final concentration of 14.0 mg/kg and 7.0 mg/kg, respectively. All other reagents and chemicals used in the study were of analytical grade.

Animals

C57BL/6 mice (3–5 months of age; n = 60) were purchased from the Beijing HFK Bioscience Co., Ltd [15]. Sig-1R knockout (Sig-1R KO) mice on a C57BL/6J background (3–5 months of age; n = 20) were provided by Nanjing Med University and characterized in the experiment as previously described [16]. We identified the genotype of the Sig-1R KO mice by PCR. Half of the mice in each group of this study were male, and half were female. The mice were housed under controlled temperature (23 ± 1 °C) and humidity (50 ± 5%) conditions and a 12-h light/dark cycle. Standard food and water were provided ad libitum. All animal procedures were performed based on the People's Republic of China legislation and the guidelines of the committee of Animal Experiments at Shenyang Pharmaceutical University.

Experimental design

In this experiment, mice under chloral hydrate (300 mg/kg, i.p.) anesthesia were subjected to BCCAO. In brief, after creating a lateral neck incision, brain ischemia was induced by occluding two carotid arteries for 15 min with microaneurysm clips [17]. Sham mice underwent the same procedure without the occlusion. In the first scheme, the wild type (WT) and Sig-1R KO mice were randomly divided into four groups: WT-Sham, Sig-1R KO-Sham, WT-BCCAO and Sig-1R KO-BCCAO (n = 4). In the second scheme, the C57BL/6 mice were randomly divided into the five groups: WT-Sham, WT-BCCAO, WT-BCCAO + PRE084 (7 mg/kg), WT-BCCAO + BD1047 (14 mg/kg) and WT-BCCAO + PRE084 (7 mg/kg) + BD1047 (14 mg/kg) (n = 5). For detecting protein expression levels, mice injected (i.p.) with PRE084, BD1047, PRE084 + BD1047 and saline every 12 h (two times) were sacrificed at 24 h after BCCAO. Mice injected (i.p.) with drugs or saline once daily for 3 consecutive days were sacrificed at 72 h after BCCAO for observation of cellular morphology. The experimental schedule is shown in Fig. 1.

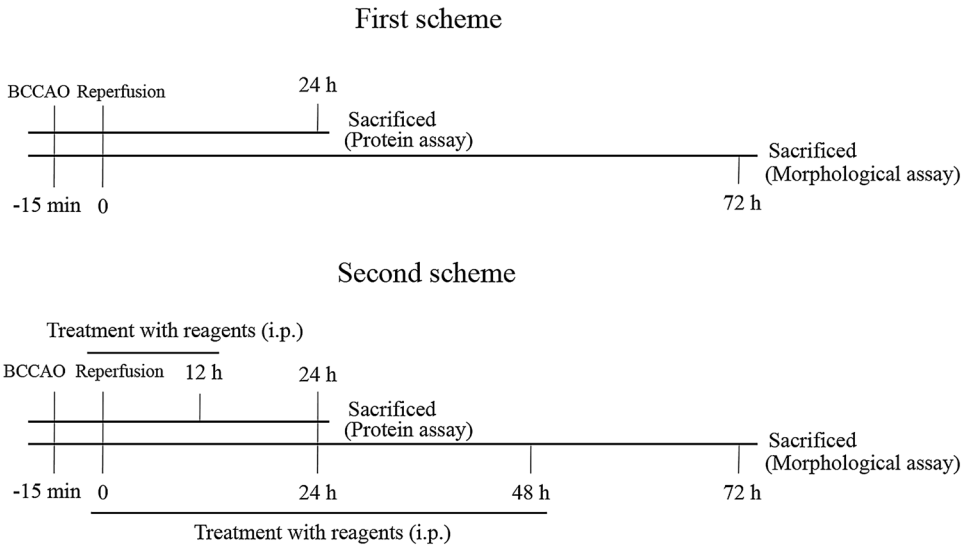

Fig. 1 Experimental protocol. Reagents: PRE084 (7.0 mg/kg), BD1047 (14.0 mg/kg), and saline (90.0 mg/kg)

TUNEL staining

To investigate the role of Sig-1R in suppressing cell death, TUNEL staining was performed on paraffin sections using Fluorescein (Roche Diagnostics, 11684795910) and an In Situ Cell Death Detection Kit (Roche Diagnostics, 11684817910) as previously described [18, 19]. For fluorescence measurements (n = 4), the sections from each group were incubated with proteinase K for 10 min at 37 °C and washed with PBS (0.1 M, pH 7.4) for 5 min. The sections were incubated in a TUNEL reaction mixture for 1 h at 37 °C and stained with DAPI for 5 min at room temperature. For non-fluorescent observations (n = 5), slides were incubated for 30 min at 37 °C with conver-POD. After washing with PBS, diaminobenzidine was used to visualize the signals. The number of TUNEL-positive cells was counted, and the data are expressed as the ratio of TUNEL-positive cells/field. Images were captured using a Nikon C2 Plus system (Japan).

Transmission electron microscopy (TEM) analysis

TEM was used to observe the ultrastructure of neurons [20]. The mice (n = 2/group) were anaesthetized with chloral hydrate (300 mg/kg, i.p.). Then, normal saline was injected into the left cardiac ventricle until colorless perfusion fluid was obtained from the right atrium. A mixture of 2.5% glutaraldehyde in 0.01 M PBS (temperature 4 °C, pH 7.4) was used as a fixative. The segments of fresh brain tissue were immediately removed and post-fixed by immersion in the fixative for 24 h at 4 °C. Then, ultrathin sections (1 mm^3) of cerebral cortical tissue were prepared following standard procedures. The ultrastructural changes of neurons were observed by TEM and imaged (JEOL H-7650).

Western blot

We analyzed the effect of Sig-1R against the expression of proteins involved in BCCAO-induced ER stress by Western blot, as previously described [21, 22]. Protein concentrations were analyzed using a BCA kit (Sigma, CA, USA). Equal amounts of protein extracts were separated in a 10% gel by SDS-PAGE. Blocking was performed for 2 h with 5% skim milk in PBS at room temperature. Then, the membrane was incubated with primary antibody against CHOP (1:400; Cell Signaling Technology, MA), p-eIF2α (1:500; Cell Signaling Technology, MA), eIF2α (1:1500; Cell Signaling Technology, MA), ATF4 (1:600; Proteintech, China), JNK (1:600; Proteintech, China), p-JNK (1:500; Cell Signaling Technology, MA), Cleaved-caspase-3 (C-caspase-3, 1:1000; Cell Signaling Technology, MA), Cleaved-caspase-12 (C-caspase-12, 1:500; Cell Signaling Technology, MA), IRE (1:500; Cell Signaling Technology, MA), p-IRE (1:800; Abcam, USA), PERK (1:200; Cell Signaling Technology, MA), p-PERK (1:250; Cell Signaling Technology, MA), NeuN (1:3000; Abcam, USA), PUMA (1:3000; Proteintech, China), Bim (1:600;Cell Signaling Technology, MA), Bcl-2 (1:1000, Santa Cruz, USA) and β-actin (1:400; Santa Cruz, USA) overnight at 4 °C, followed by incubation with secondary antibody against rabbit or mouse IgG (Santa Cruz, USA) for 2 h at room temperature.

Statistical analysis

All statistical analyses were performed using SPSS 19.0 software (IBM, USA). Data are presented as the mean ± SD and were analyzed by one-way ANOVA followed by post hoc tests. A value of $p < 0.05$ was considered statistically significant.

Results

Sig-1R KO promoted neuronal apoptosis in the cerebral cortex of BCCAO mice

To investigate the effect of Sig-1R against ER stress-mediated apoptosis in BCCAO mice, we detected the neuronal apoptosis in the cerebral cortex Sig-1R KO mice after BCCAO. Herein, the Sig-1R-induced anti-apoptotic effect was investigated using TUNEL staining. TUNEL-positive staining (green) was significantly increased in WT-BCCAO mice compared with WT-Sham mice and significantly increased in Sig-1R KO-BCCAO mice compared with WT-BCCAO mice (Fig. 2a). WT-Sham mice showed comparatively complete nuclear structures, while WT-BCCAO mice displayed chromatin condensation and chromatin accumulation along the inside of the nuclear membrane. Sig-1R KO-BCCAO mice showed more severe nuclear damage, such as nuclear membrane dissolution and nuclear chromatin spillover, compared with WT-BCCAO mice (Fig. 2b). We further found that the expression of NeuN was significantly decreased in Sig-1R KO-BCCAO mice compared with WT-BCCAO mice (Fig. 2c). These results indicate the presence of significantly increased neuronal apoptosis in BCCAO mice with Sig-1R KO.

Sig-1R activation decreased neuronal apoptosis in the cerebral cortex of WT-BCCAO mice

To further confirm the effect of Sig-1R against ER stress-mediated apoptosis, we examined WT-BCCAO mice following treatment with the Sig-1R agonist PRE084, the Sig-1R antagonist BD1047 or PRE084 + BD1047. The results indicated that TUNEL-positive staining (brown) was increased in WT-BCCAO mice compared with WT-Sham mice and in WT-BCCAO mice compared with PRE084-treated WT-BCCAO mice (Fig. 3a). Less nuclear damage was observed in WT-Sham mice and PRE084-treated WT-BCCAO mice than in WT-BCCAO mice (Fig. 3b). Meanwhile, the expression of NeuN was significantly increased in PRE084-treated WT-BCCAO mice compared with WT-BCCAO mice (Fig. 3c). However, all the protective effects of PRE084 could be inhibited by BD1047 (Fig. 3a-c). These results

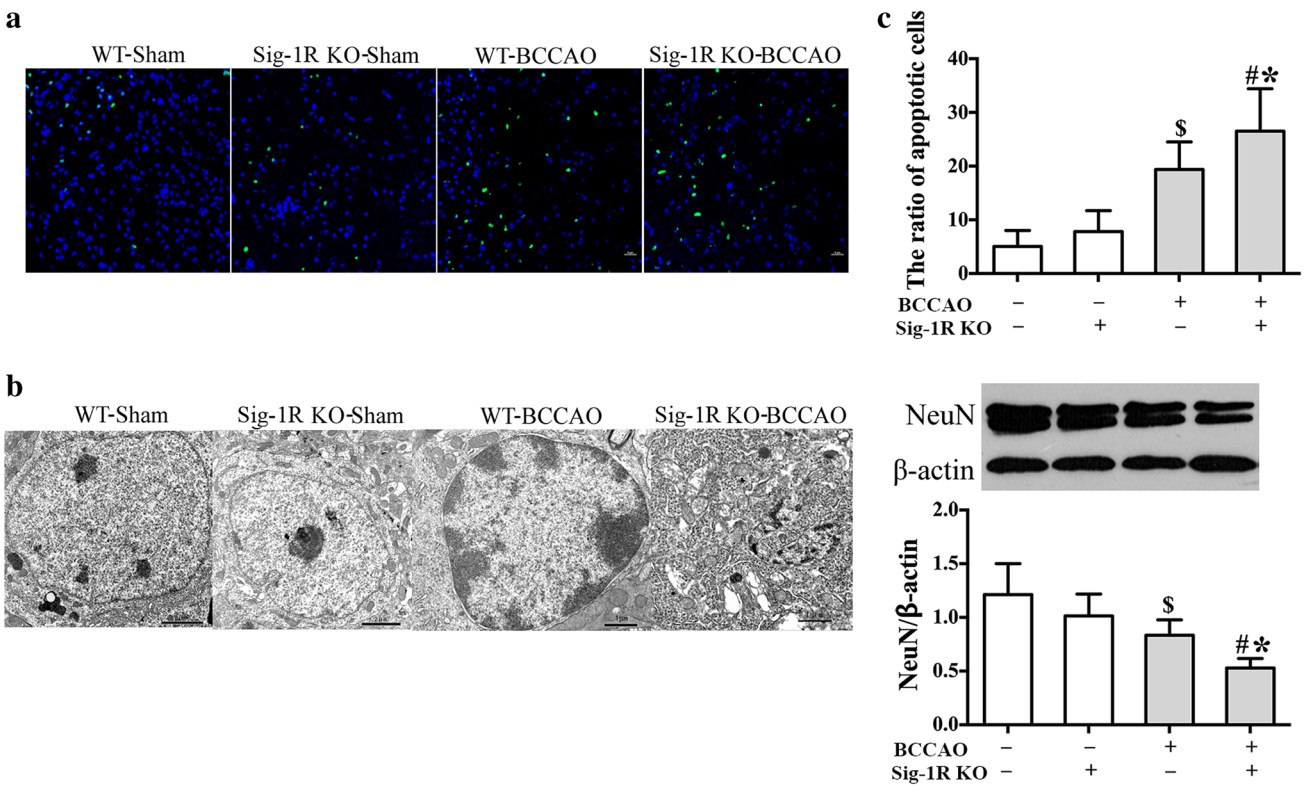

Fig. 2 Sig-1R KO promoted neuronal apoptosis in the cerebral cortex of BCCAO mice. **a** The figures and quantified results show apoptotic cells identified by TUNEL staining in each group. Nuclei were counterstained with DAPI. TUNEL and DAPI images were merged. Scale bar = 10 μm. **b** Electron microscopy images of neuronal nuclei in the cerebral cortex of mice. **c** Western blot bands and quantification of NeuN expression in the cerebral cortex of mice. Images shown are representative of three independent experiments. Data are presented as the mean ± SD (n = 4). $^{\$}p < 0.05$ vs. WT-Sham; $^{\#}p < 0.05$ vs. KO-Sham; $^{*}p < 0.05$ vs. WT-BCCAO

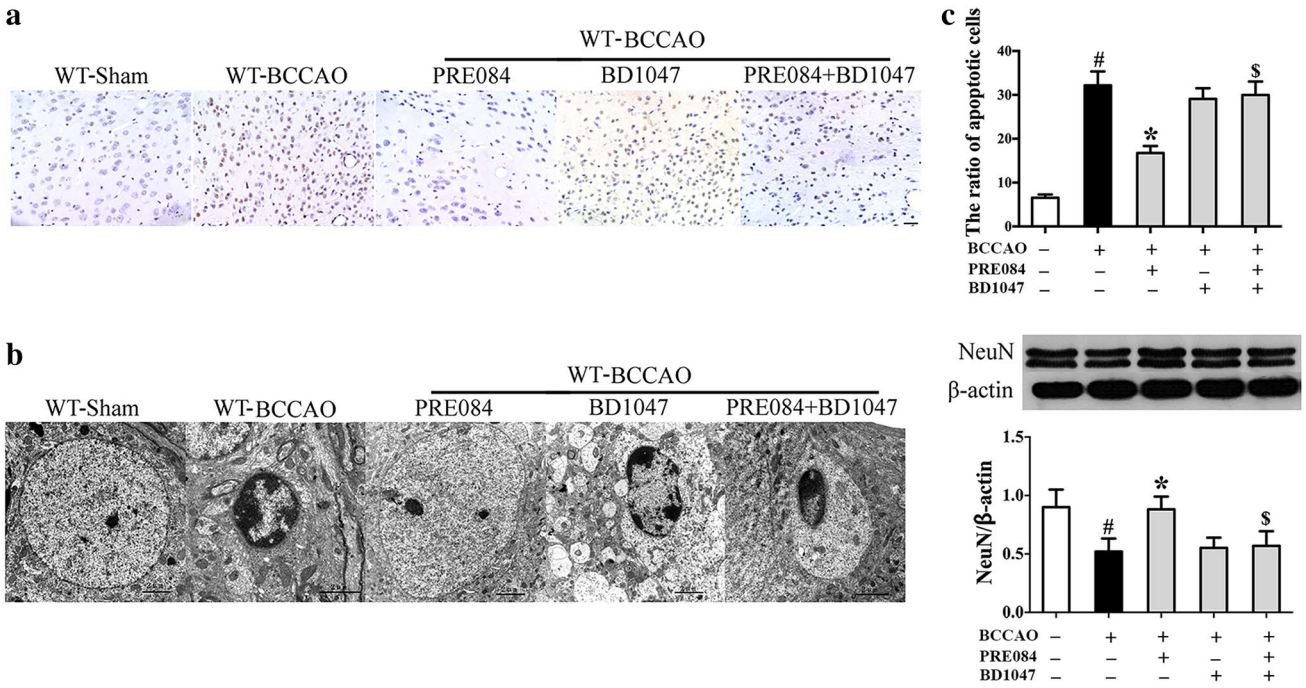

Fig. 3 Sig-1R activation alleviated neuronal apoptosis in the cerebral cortex of WT-BCCAO mice. **a** Activation or inhibition of Sig-1R in response to apoptosis was detected and quantified by TUNEL staining. Merged brown and blue nuclei indicate TUNEL-positive cells. Scale bar = 20 μm. **b** Electron microscopy images showing that the treatment of mice with PRE084, BD1047 or PRE084 + BD1047 influenced the ultrastructure of nuclei in C57BL/6 mice subjected to 15-min BCCAO. **c** Western blot bands and quantification of NeuN expression in the cerebral cortex of WT-BCCAO mice after treatment with PRE084, BD1047 or PRE084 + BD1047. Images shown are representative of three independent experiments. Data are presented as the mean ± SD (n = 4). $^{\#}p < 0.05$ vs. WT-Sham; $^{*}p < 0.05$ vs. WT-BCCAO; $^{\$}p < 0.05$ vs. PRE084 + BCCAO

suggest that Sig-1R activation protected WT-BCCAO mice against neuronal apoptosis.

Sig-1R regulated expression of ER stress-mediated CHOP pathway proteins in the cerebral cortex of WT-BCCAO mice

It has been demonstrated that Sig-1R expression in response to ER stress is mediated by the CHOP pathway, as one of the cellular responses to ER stress [23]. Meanwhile, neuronal apoptosis is mediated by the ER stress pathway in WT-BCCAO mice [17]. An interesting line of inquiry is to determine whether Sig-1R has a protective effect against ER stress-mediated CHOP pathway activation in WT-BCCAO mice. PERK/p-eIF2α/ATF4 regulates CHOP expression during ER stress in WT-BCCAO mice [24]. We found that the protein expression of p-PERK, p-eIF2α, ATF4, and CHOP was significantly increased in WT-BCCAO mice compared with WT-Sham mice (Fig. 4b–e). However, the expression of these proteins showed a more significant increase in Sig-1R KO-BCCAO mice than in WT-BCCAO mice (Fig. 4b–e). Then, we treated WT-BCCAO mice with PRE084, BD1047 and PRE084 + BD1047 to further evaluate the protective effect of Sig-1R (Fig. 5a). WT-BCCAO mice treated with PRE084, activating Sig-1R, showed significantly decreased protein expression of p-PERK, ATF4 and CHOP compared with WT-BCCAO mice, while this decrease was significantly reversed by the Sig-1R antagonist BD1047 (Fig. 5b–d). In addition, WT-BCCAO mice treated with BD1047 alone showed no significant differences in CHOP pathway regulating compared with WT-BCCAO mice (Fig. 5b–d). These results indicate that the induction of Sig-1R inhibited ER stress-mediated apoptosis via the CHOP pathway in WT-BCCAO mice.

Sig-1R regulated the expression of ER stress-mediated JNK and caspase-12 pathway-related proteins in the cerebral cortex of WT-BCCAO mice

To further investigate the mechanisms responsible for the protective effects of Sig-1R against ER stress in WT-BCCAO mice, we examined the functional role of Sig-1R in the JNK and caspase-12 pathways. IRE1 is an upstream activator of both JNK and caspase-12 [25]. At 24 h after cerebral I/R, the levels of p-IRE1, p-JNK, Bim, PUMA, C-caspase-12 and C-caspase-3 were significantly increased while the level of the anti-apoptotic factor Bcl-2 was significantly

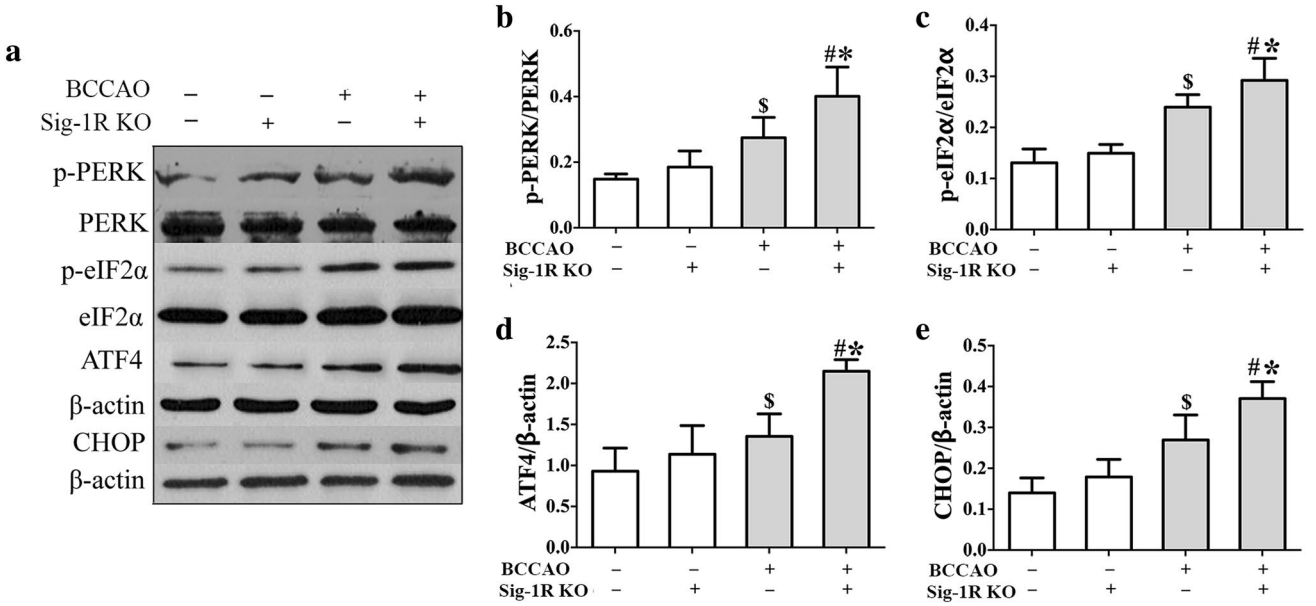

Fig. 4 Sig-1R KO induced ER stress-mediated CHOP signaling protein activation in the cerebral cortex of WT-BCCAO mice. Western blot bands **(a)** and quantification were used to measure the protein levels of p-PERK (**b**), p-eIF2α (**c**), ATF4 (**d**) and CHOP (**e**). Data are presented as the mean ± SD (n = 4). $^{\$}p < 0.05$ vs. WT-Sham; $^{\#}p < 0.05$ vs. KO-Sham; $^{*}p < 0.05$ vs. WT-BCCAO

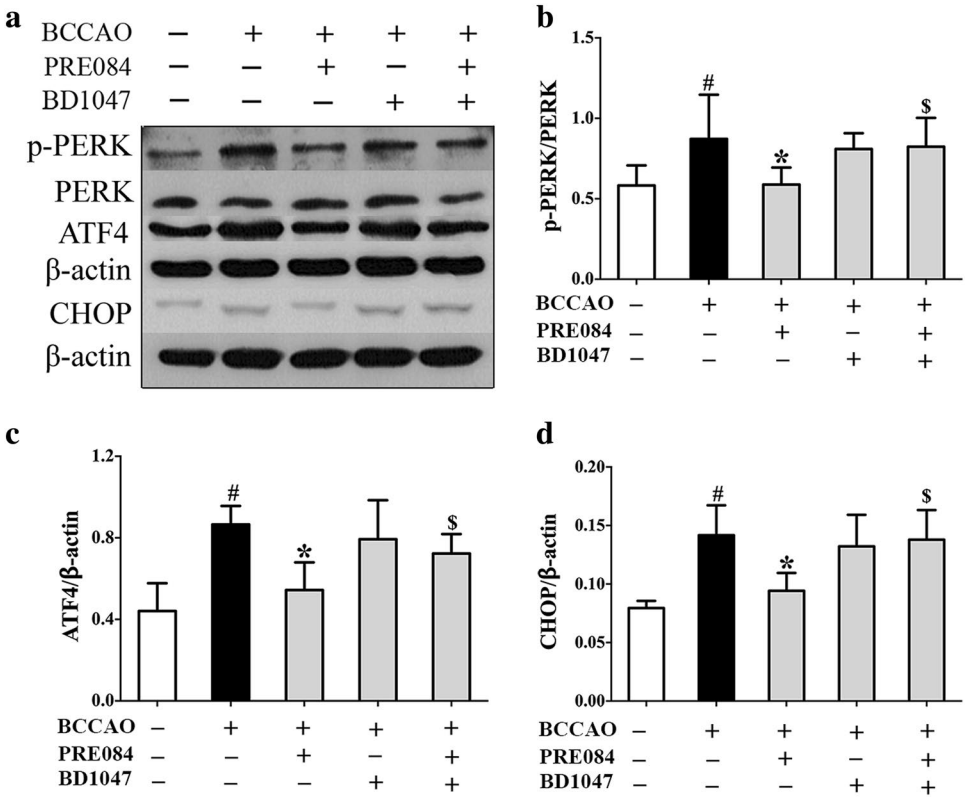

Fig. 5 Sig-1R activation rescued the expression of ER stress-mediated CHOP pathway-related proteins in the cerebral cortex of WT-BCCAO mice. Western blot bands (**a**) and quantification of p-PERK (**b**), ATF4 (**c**), CHOP (**d**) protein levels in each group. Data are presented as the mean ± SD (n = 5). $^{\#}p < 0.05$ vs. WT-Sham; $^{*}p < 0.05$ vs. WT-BCCAO; $^{\$}p < 0.05$ vs. PRE084 + BCCAO

decreased in Sig-1R KO-BCCAO mice compared with WT-BCCAO mice (Fig. 6b–h). In addition, Sig-1R activation with PRE084 significantly attenuated the expression of ER stress-mediated JNK and caspase-12 pathway-related proteins, whereas treatment with the Sig-1R antagonist BD1047 reversed the protective effect of Sig-1R activation

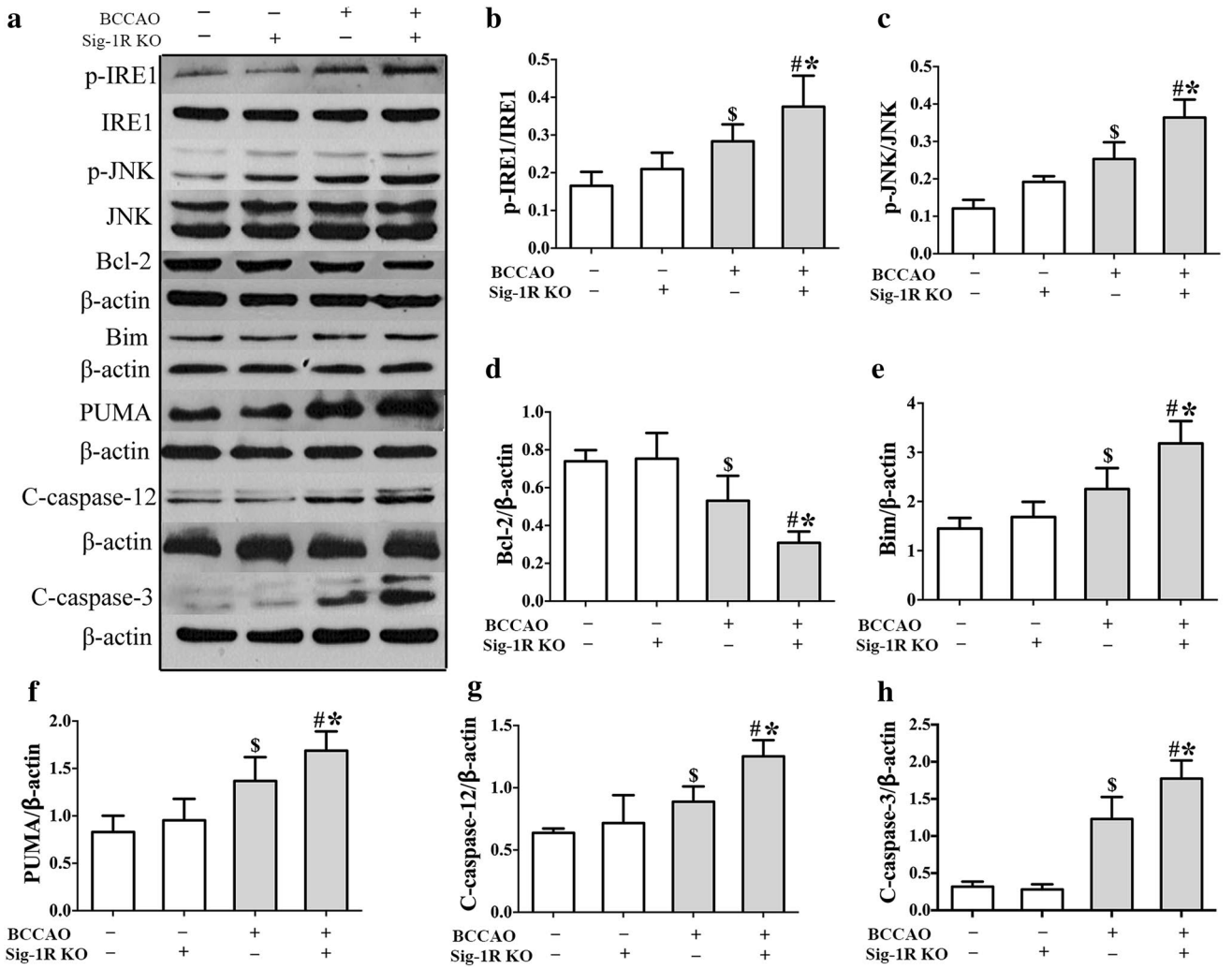

Fig. 6 Sig-1R KO increased the abnormal expression of ER stress-mediated JNK and caspase-12 pathway-related proteins in the cerebral cortex of WT-BCCAO mice. Western blot bands (**a**) and quantification of p-IRE1 (**b**), p-JNK (**c**), Bcl-2 (**d**), Bim (**e**), PUMA (**f**), C-caspase-12 (**g**), and C-caspase-3 (**h**) protein levels in the cerebral cortex of mice. Data are presented as the mean ± SD (n = 4). $^{\$}p < 0.05$ vs. WT-Sham; $^{\#}p < 0.05$ vs. Sig-1R KO-Sham; $^{*}p < 0.05$ vs. WT-BCCAO

by PRE084 (Fig. 7b–h). The treatment of WT-BCCAO mice with BD1047 alone showed no effect on the JNK and caspase-12 pathways. These results suggest that Sig-1R exerted a protective effect against the ER stress-mediated JNK and caspase-12 pathways in WT-BCCAO mice.

Discussion

Our previous study showed that Sig-1R activation inhibits neuronal loss in mice with cerebral I/R injury [26]. In addition, the induction of Sig-1R led to a decrease in the cell death induced by ER stress [27]. ER stress can be induced in mice subjected to BCCAO for 15 min [17]. Consequences of prolonged ER stress are neuronal apoptosis activation and morphological cell damage [28–30]. In the current study, our results revealed significantly more TUNEL-positive neurons and more severe nuclear damage in Sig-1R KO-BCCAO mice than in WT-BCCAO mice. To further verify the effect of Sig-1R, we treated WT-BCCAO mice with the Sig-1R agonist PRE084 alone and together with the antagonist BD1047 to detect changes in the related indicators. The results showed that Sig-1R activation decreased the number of TUNEL-positive neurons and improved nuclear morphology, whereas the treatment of WT-BCCAO mice with BD1047 inhibited the protective effect of PRE084. These results indicate that Sig-1R activation improved neuronal morphological changes and reduced neuronal apoptosis in WT-BCCAO mice. In this study, we report for the first time that Sig-1R can protect neurons against ER stress-mediated apoptosis in mice with cerebral I/R injury induced by BCCAO (Fig. 8).

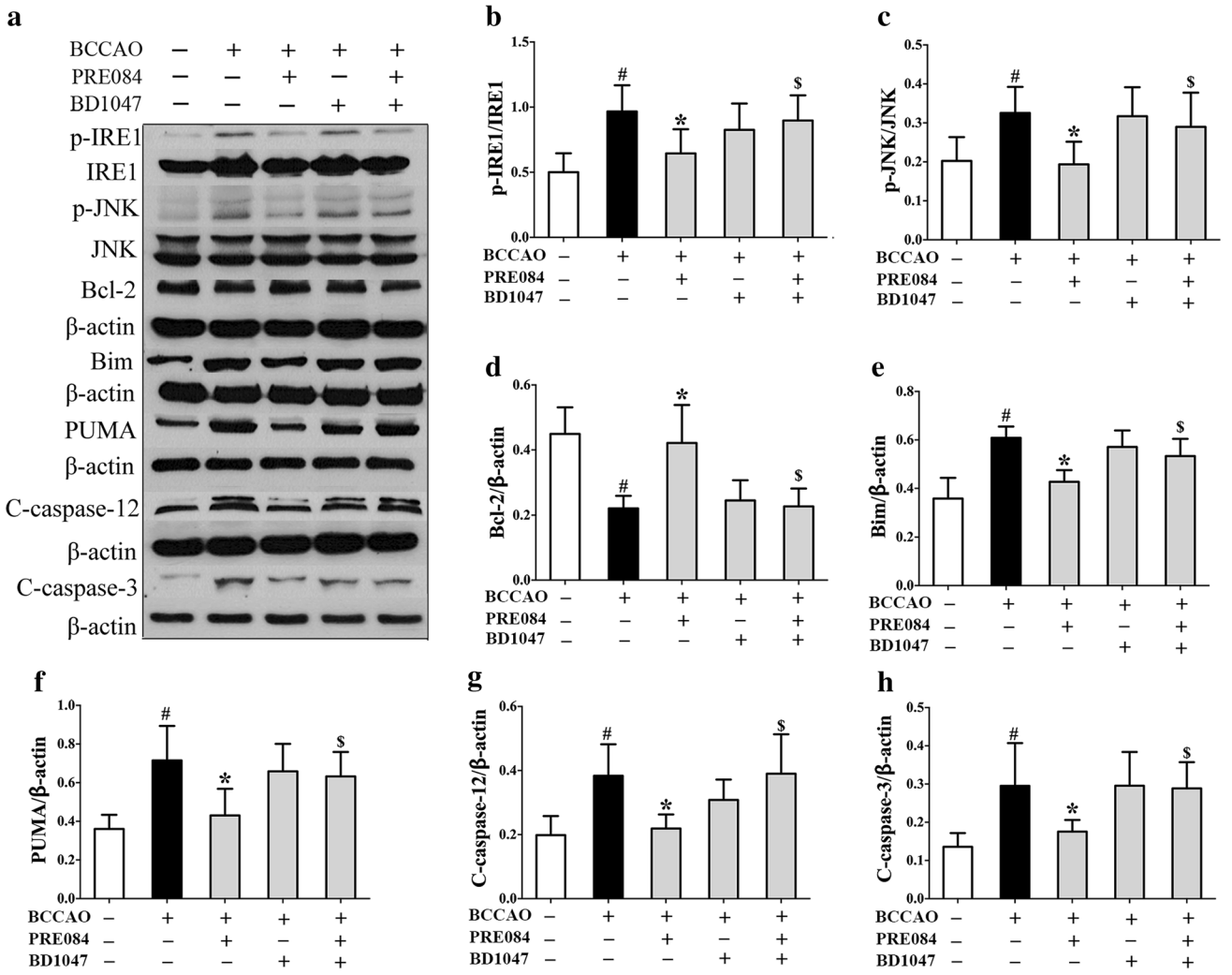

Fig. 7 Sig-1R activation inhibited the abnormal expression of ER stress-mediated JNK and caspase-12 pathway-related proteins in the cerebral cortex of WT-BCCAO mice. Western blot bands (**a**) and quantification of p-IRE1 (**b**), p-JNK (**c**), Bcl-2 (**d**), Bim (**e**), PUMA (**f**), C-caspase-12 (**g**) and C-caspase-3 (**h**) protein levels in the cerebral cortex of WT-BCCAO mice treated with PRE084, BD1047 or PRE084 + BD1047. Data are presented as the mean ± SD (n = 5). $^{\#}p < 0.05$ vs. WT-Sham; $^{*}p < 0.05$ vs. WT-BCCAO; $^{\$}p < 0.05$ vs. PRE084 + BCCAO

When conditions of stress are prolonged or excessive, the UPR fails to restore the ER homeostasis, and then the apoptotic signaling pathways will be activated [31]. UPR signaling switches cells from a pro-survival mode to a pro-apoptosis mode through the transcriptional induction of CHOP and the activation of the JNK and caspase-12-dependent pathways [25]. CHOP is an important transcription factor in the response to ER stress-mediated apoptosis, and CHOP is regulated by all three branches of UPR signaling [32, 33]. The expression of CHOP can be regulated by PERK-eIF2-ATF4 [34]. CHOP serves as a pivotal stimulus for cell death, and it is mainly induced by ATF-4. The activation of PERK enhances the translation of ATF4, subsequently increasing the expression of CHOP, which induces apoptosis [35]. Sig-1R knockdown promotes apoptosis induced by ER stress in cultured CHO cells [36]. However, whether the Sig-1R-mediated decrease in neuronal apoptosis in BCCAO mice is related to ER stress remains to be investigated. Our data showed that the protein levels of p-PERK, p-eIF2α, ATF4 and CHOP were significantly increased in Sig-1R KO-BCCAO mice. The activation of Sig-1R with PRE084 significantly decreased these protein levels compared with those of WT-BCCAO mice, and the effects of PRE084 were blocked by the Sig-1R antagonist BD1047. These results indicate that the activation of Sig-1R inhibited ER stress-mediated apoptosis through the CHOP pathway in mice with cerebral I/R injury.

Phosphorylated JNK and activated caspase-12 are also involved in prolonged or excessive ER stress [37]. IRE1 contributes to ER stress-mediated apoptosis through the

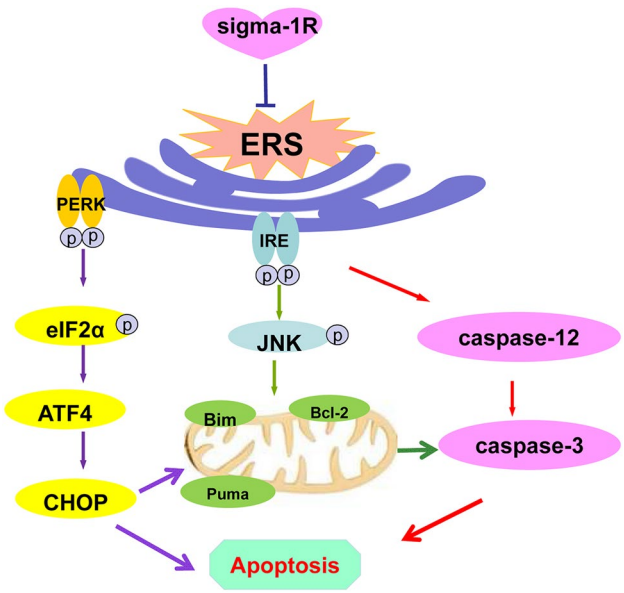

Fig. 8 Summary of the possible mechanisms underlying the protective effect of Sig-1R against ER stress-mediated apoptosis in mice with cerebral I/R injury

JNK and caspase-12 pathways [38]. JNK and CHOP activation decreases anti-apoptotic protein Bcl-2 expression and increases expression of the pro-apoptotic proteins PUMA and Bim to induce apoptosis in I/R-induced ER stress [25]. Caspase-12 is located in the ER and normally exists in an inactive pro-caspase form. When activated by ER stress, IRE1 interacts with caspase-12 to induce its cleavage and activation [39, 40]. Activated caspase-12 cleaves caspase-9, which further cleaves caspase-3 to initiate ER-mediated apoptosis [41]. A caspase-12 deficiency has been shown to inhibit ER stress-mediated apoptosis [42]. In this study, Sig-1R KO-BCCAO mice showed a significant increase in the protein levels of p-IRE1, p-JNK, Bim, PUMA, C-caspase-12 and C-caspase-3 and a decrease in the protein level of Bcl-2 compared with WT-BCCAO mice. Moreover, WT-BCCAO mice treated with PRE084 showed decreased protein levels of p-IRE1, p-JNK, Bim, PUMA, C-caspase-12 and C-caspase-3 and an increased protein level of Bcl-2 compared with WT-BCCAO mice. The effects of PRE084 were blocked by the Sig-1R antagonist BD1047. These results suggested that Sig-1R exerted a neuroprotective effect against ER stress-mediated apoptosis through inhibiting the JNK and caspase-12 pathways. Therefore, our results suggest that Sig-1R activation inhibited ER stress-mediated apoptosis through the JNK and caspase-12 pathways in mice with cerebral I/R injury. We infered an upregulation in ERS-related proteins in response to the Sig-1R antagonist BD1047. However, we found that BD1047 treatment did not significantly alter the expression of ERS-related proteins in WT-BCCAO mice. The reason for these results of the experiment also might be explained by the limitation of the dosage or treatment duration of BD1047.

In conclusion, the results of the present study indicate that Sig-1R protected neurons against ER stress-mediated apoptosis in mice with cerebral I/R injury and that the mechanism underlying this process might be related to the inhibition of the CHOP, JNK and caspase-12 pathways. These findings may lead to novel therapeutic strategies for cerebral I/R injury.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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