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Resveratrol Attenuates Endoplasmic Reticulum Stress-Induced Cell Death and Results in Functional Improvement after Traumatic Brain Injury in Mice

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Abstract

Resveratrol (RSV), a polyphenol antioxidant, has been reported to function as a neuroprotector. We explored the molecular mechanisms that underlines the anti-inflammatory activity of RSV in traumatic brain injury (TBI) in mice relevant to endoplasmic reticulum stress (ERS). By establishing three experimental groups (sham, TBI, and TBI+RSV), we examined the effects of RSV after TBI on ERS and caspase-12 apoptotic pathways cascades. The protein expressions levels of C/EBP homologous protein (CHOP), glucose regulated protein 78kD (GRP78), caspase-3, and caspase-12 in cortical brain tissues were evaluated by western blotting. The cortical brain tissues were also subjected to a qPCR analysis of mRNA expression of tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Additionally, immunofluorescence staining was used to determine the expression of GRP78 in microglia (ionized calcium binding adaptor molecule 1;Iba-1) and neurons (neuronal nuclei;NeuN). The neurological function of the mice was analyzed based on modified neurological severity scores (mNSS). After RSV treatment, the expression of CHOP, GRP78, caspase-3 and caspase-12 decreased, and qPCR results showed downregulation of TNF-α and IL-1β. Immunofluorescence stain demonstrated Iba-1^{+}/$GRP78^{+}$ and $NeuN^{+}$/$GRP78^{+}$ cells decreased after RSV treatment. The mNSS analysis indicated improvement following RSV treatment. RSV alleviated neuro-apoptosis by inhibiting the ERS signaling pathway and also improved the neurological function of mice with TBI.

Keywords: Traumatic brain injury; Resveratrol; Endoplasmic reticulum stress; Apoptosis; Neuroinflammation

1. Introduction

TBI is a major cause of mortality and impairment among individuals aged <45 years which is caused by insult to the brain as a result of an external mechanical force [1]. At the basic level, TBI is categorized by its primary and secondary phases. The primary phase occurs at the moment the external force disrupts the brain, whereas the secondary insult takes a longer period of time to develop and involve a multitude of cellular processes that lead to detrimental effects on brain tissues [2]. Neuroinflammation is involved in the secondary events after TBI, and it has positive and negative effects , i.e., enhancing neurorepair as well as promoting

secondary brain injury[3]. In the central nervous system (CNS), the microglial cells play critical roles in the inflammation after TBI[4]. CNS microglia are important for the phagocytosis of cellular particles from injury sites, in addition to being regulators of cell survival, cell death, and inflammation[5].

Secondary brain injury can lead to severe neurological deficits by processes that involve ERS, inflammation, apoptosis, and several other mechanisms[6]. ERS results in the abnormal accumulation of unfolded proteins, which changes the endoplasmic reticulum (ER) environment, resulting in decreased quantities of functional proteins[7]. ERS is a protective process during cellular stress, but long-term ERS causes a variety of pathological processes including inflammation and CHOP-dependent apoptosis[8]. Recent studies have found that ERS is highly correlated with apoptosis in many CNS diseases[9]. Changes in the ER-regulated protein folding pathway can result in increased synthesis of reactive oxygen species (ROS), which causes misfolded protein accumulation. A moderate unfolded protein response (UPR) during brain injury can help maintain cell function and cell survival, but the accumulation of apoptotic factors and proinflammatory cytokines during sustained ERS can increase brain cell death [10]. In TBI, ERS is increased, and reducing this response may reduce secondary neuronal damage[6].

RSV is a naturally occurring phytoalexin that can be isolated from various plants such as grape and hellebore which is also thought to have antagonistic effects on inflammation. Recently, researchers have demonstrated that RSV exhibits strong protection against TBI, metabolic disease, and other age-related diseases [11]. In animal experiments, RSV has been shown to cross the blood–brain barrier (BBB), and it is thought to be potentially useful for the treatment of several neurodegenerative and neuroinflammatory CNS disease [12]. Although experiments involving RSV have led to major improvements in neuroinflammation after TBI[13], the cellular mechanisms underlying RSV anti-inflammatory effects in TBI is have not been completely elucidated.

In this study, we explored the fundamental cellular processes underlying the anti-inflammatory actions of RSV in vivo and determined whether the protective effects involve ERS.

2. Materials and Methods

2.1 Animals and groups

C57BL/6 mice weighing 24-28g were obtained from the Shanghai Laboratory Animal Centre, Chinese Academy of Sciences (SLACCAS; Shanghai, China). In a temperature-controlled room the mice were given food and water, and the room light stimulated normal day and night changes. The study was approved by the Animal Experimental Center of Wenzhou Medical University and was conducted under its supervision. Using computer-generated random numbers, 54 mice were randomized into three groups: a sham operation group (n = 18), a TBI group (n = 18), and a TBI group treated with RSV (TBI+RSV, n = 18). All experiments were performed in a blinded manner.Altogether we lost 3 of the experimental animals :2 from over dosage of anaesthesia in each of the groups (sham and TBI+RSV) and 1 from the TBI group due to higher impact.

2.2 TBI

The mice were intraperitoneally anesthetized with 10% chloride hydrate (3.0 ml/kg, intraperitoneally) before creating the controlled cortical impact(CCI) model. The mice were then fixed using ear bars in a stereotaxic device (KOPF, Tujunga, CA, USA). Surgery began with a scalp incision at the midline, followed by a 3.0-mm craniotomy in the center of the parietal bone on the right side. The desired injury (moderate TBI) was induced using Impact One^{TM} Stereotaxic Impactor for CCI (Leica, USA), positioned in contact with the top of the exposed dura mater, and set to the dura mater using the following impact parameters: depth 2.0 mm, velocity 4.5 m/s, and duration time 200ms, as previously reported. Mice with a dura mater herniation were excluded from the study. After successful cortical impact, the craniotomy site was sterilized and the scalp was sutured. The mice were returned to a ventilated cage at 37 °C, where they regained consciousness. The sham group mice underwent the same surgical interventions without cortical impact.

2.3 RSV administration.

RSV (R5010, sigma, USA) were purchased, and diluted with dimethyl sulfoxide (DMSO)/normal saline. TBI+RSV mice were treated with an intraperitoneal RSV injection (40 mg/kg) immediately after establishment of the TBI model and then daily up to 3 d after TBI.

2.4 Western blot analysis

The brain tissues from the injured cortex were placed for 30 min in ice-cold radioummunoprecipitation assay (RIPA) lysis buffer with phenylmethanesulfonyl fluoride (PMSF) (RIPA: PMSF=100:1). A bicinchoninic acid (BCA) protein assay kit (Beyotime Biotechnology, Haimen, China) was used to assess the protein concentration. The proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane, followed by blocking with 5% milk for 2 h and then overnight incubation with the following primary antibodies: anti-GRP78 monoclonal antibody (1:500; rabbit, ab21685, Abcam), anti-CHOP antibody (1:1000; rabbit, ab179823, Abcam), anti-caspase-12 polyclonal antibody (1:500; rabbit, ab62484, Abcam), and anti-caspase-3 polyclonal antibody (1:750; rabbit, ab13847, Abcam). The secondary antibody was horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:2000; CST, Beverly, MA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:1000; 5174; CST) was used as a normalization marker for all samples. SeeBlue®Plus2 Pre-Stained Standard (Life Technologies, Carlsbad, CA, USA) was used to determine the molecular weights. The secondary antibody was visualized using LumiGLO chemiluminescent substrate (CST) following the manufacturer's directions. The bands were scanned and analyzed using optical density values with background deductions and normalized to GAPDH using NIH ImageJ software.

2.5 Immunofluorescence

To asses ERS in neurons and microglia, brain sections were processed for immunostaining with antibodies against ionized calcium-binding adapter molecular 1 (Iba1; 1:800; mouse, ab5076, Abcam), neuronal nuclei (NeuN; 1:150; mouse, NBP1-92693, Novus), and GRP78 (1:500; rabbit, ab21685, Abcam), which are microglial, neuronal, and ERS markers, respectively. First, sections were soaked in paraformaldehyde and then rinsed with PBS three times every 5min. This was followed by blocking with 5% goat serum albumin (Sigma) + 0.4% Triton-X for 1 h at room temperature, followed by incubation with primary antibody at 4°C overnight. Then, sections were washed with PBS three times and incubated with a mixture containing fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (green; 1:400, Earth) and DyLight 594 goat anti-mouse antibody (red; 1:400, Earth) for 1 h at room temperature. The sections were then washed three times with PBS and reacted with 4′,6-diamidino-2-phenylindole (DAPI; Solarbio) for 5-10 min. The sections were then examined

using a scanning fluorescence microscope (Leica Microsystems) ImageJ software was used to assess co-labeling of Iba1 or NeuN with GRP78. The statistical analysis was based on six randomly selected sections from each mouse. Six digital micrographs were randomly taken at the margins of the hematoma in each section. The number of double-positive cells were counted and the mean number per section for each brain was calculated.

2.6 Quantitative real-time PCR

The injured cortex was separated from the brain. The total mRNA was then extracted using Trizol (15596018; Thermo Fisher). Reverse transcription of 2 μg of total mRNA to cDNA was conducted using a RevertAid First Strand cDNA Synthesis Kit (K1622; Thermo Fisher). Thereafter, SYBR Premix Ex Taq II (RR820A; Tli RNase H Plus) was used to perform real-time PCR. The relative mRNA expression levels were normalized to GAPDH. The relative mRNA expression of IL-1β, TNF-α, and GAPDH was calculated using a ΔΔCt analysis. The primers used for real-time PCR were synthesized by Invitrogen (Shanghai, China) and are presented in Table 1.

Table 1. Primers used for quantitative real-time PCR.

Gene	Forward primer 5′-3′	Reverse Primer 5′-3′
IL-1β	AATGACCTGTTCTTTGAGTTGA	TGATGTGCTGCTGCGAGATTTGAAG
TNF-α	GAAAAGCAAGCAGCCAACCA	CGGATCATGCTTTCTGTGCTC
GAPDH	GCCAAGGCTGTGGGCAAGGT	TCTCCAGGCGGCACGCAGA

2.7 Neurological deficit

Neurological impairment was assessed based on mNSS at 24, 48, and 72 h after TBI (n = 6 mice per group per time point). Experimenters were blinded to the animal groups. Each neurological test was performed twice to confirm the data. The mNSS assesment involved ten tasks to assess reflexes, balance, motor functions (abnormal movement and muscle status), and sensory functions (visual, proprioceptive, and tactile). The mNSS ranged from 0 (normal function) to 18 (maximum impairment). One point was assigned for each abnormal function or for the absence of a tested reflex. Higher scores are associated with greater neurological dysfunction. Before TBI, all mice were trained for 3 d.

2.8 Statistical Analysis

The experimental outcomes are presented as the mean ± SD. Differences in means were analyzed using non-parametric one-way and two-way analysis of variance (ANOVA) with usage of appropriate post hoc comparisons . P<0.05 was considered statistically significant.

3. Results

3.1 RSV downregulates GRP78, CHOP, caspase-3, and caspse-12 at 24 h post TBI

Western blot analysis revealed that GRP78 and CHOP expression in the TBI group increased significantly compared to the sham group and downregulated by RSV at the 24 h (Fig. 1A, 1B and 1C). Caspase-3 protein expression was significantly elevated in the TBI group compared to the sham group at 24 h, and there was a significant reduction in the TBI+RSV group compared to the TBI group (Fig. 1A and 1D). The caspase-12 expression was profoundly increased in the TBI group compared to the sham group, reversed in the TBI+RSV group (Fig. 1A and 1E).

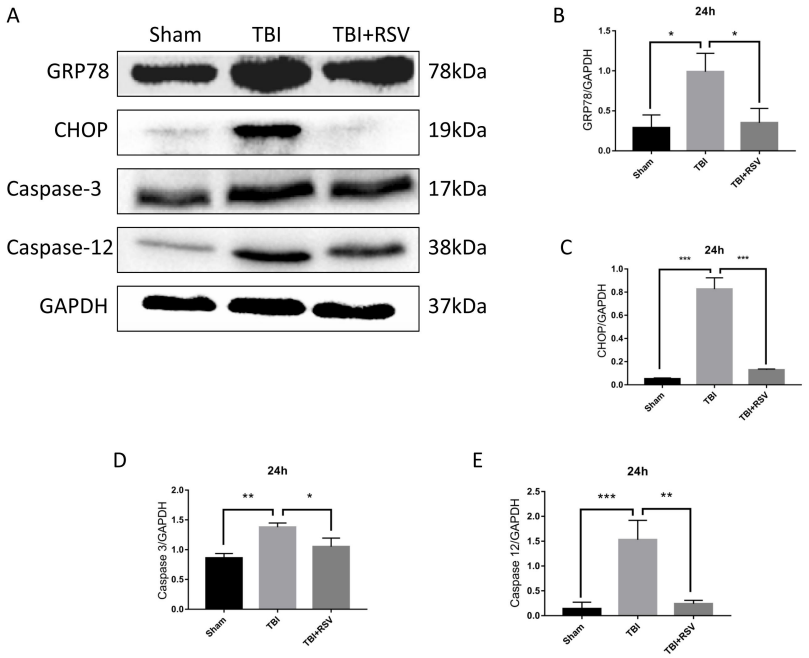

Figure 1. Western blot analysis of GRP78, CHOP, caspase-3, and caspase-12 at 24 h. GRP78, CHOP, caspase-3 and caspase-12 protein expression were significantly higher in the TBI group compared to the sham group. In comparison to the TBI group, significant decreases in these markers were recorded at 24 h in the TBI+RSV group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. n = 3 per group. RSV: resveratrol; TBI: traumatic brain injury.one-way ANOVA

3.2 RSV attenuates ERS and apoptotic pathways at 72 h post TBI

At 72 h, GRP78, CHOP, caspase-3 and caspase-12 remained significantly elevated in the TBI group compared to the sham group (Fig. 2A-E). Continuous RSV treatment for 72 h effectively decrease the expression of these signaling protein, with significant differences in CHOP ($P< 0.01$) and in GRP78, caspase-3 and caspase-12 (all $P< 0.05$) between the TBI and TBI+RSV groups (Fig. 2A-E). This indicates that persistent ERS, as confirmed by the presence of GRP78 and CHOP at 72 h, led to apoptosis, as indicated by the higher levels of caspase-3 and caspase-12, but RSV was able to partially decrease these proteins.

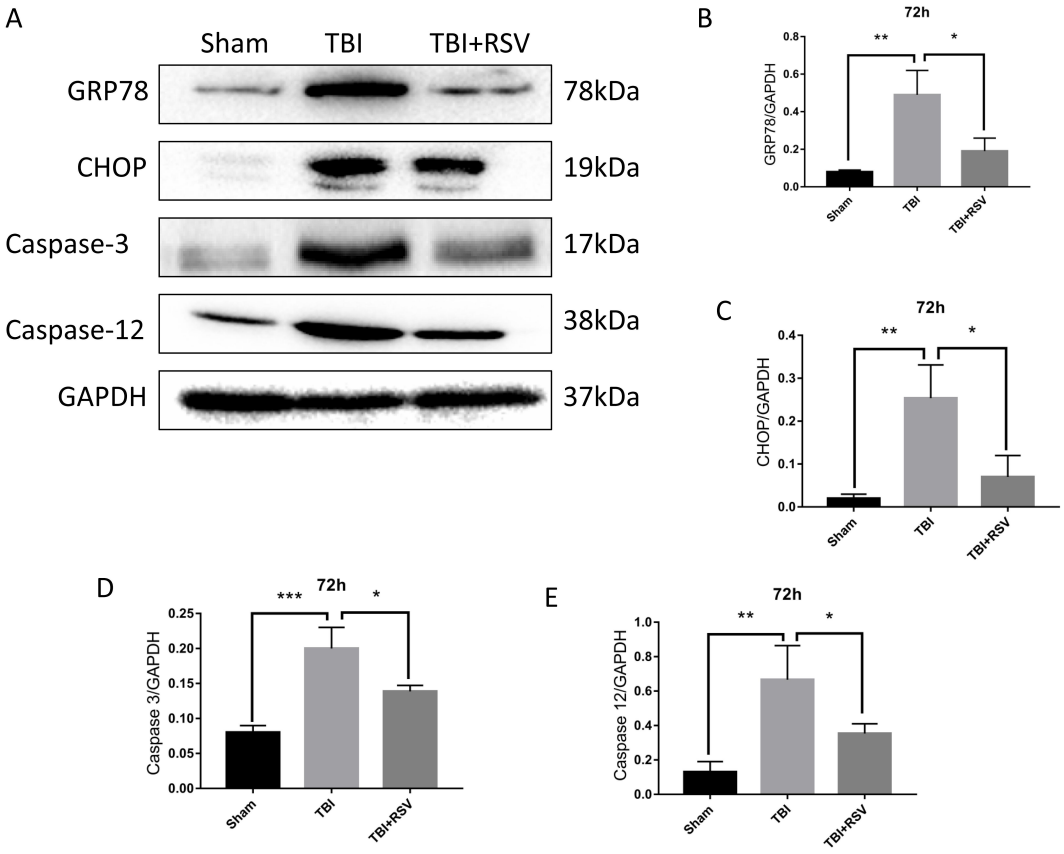

Figure 2. Western blot analysis of GRP78, CHOP, caspase-3, and caspase-12 at 72 h. GRP78, CHOP, and caspase-3 and caspase-12 protein expression was significantly increased in the TBI group compared to the sham group. In comparison to the TBI group, significant decreases in these markers were recorded at 72 h in the TBI+RSV group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. n = 3 per group. RSV: resveratrol; TBI: traumatic brain injury. one-way ANOVA

3.3 RSV decrease GRP78 immunofluorescence in primary microglial after TBI

To assess ERS in microglia in the injured cortex, we used an immunofluorescence protocol to stain Iba-1 (red) and GRP78 (green) at 24 h after TBI. As shown in Fig.3A and 3C, GRP78 immunoreactivity was localized to activated microglia in the injured cortex, and the proportion of GRP78-positive microglia was profoundly higher in the TBI group than in the sham group, in which GRP78 expression was barely visible. GRP78 expression in the microglia of the injured cortex was decreased by RSV treatment compared to the expression in the TBI group.

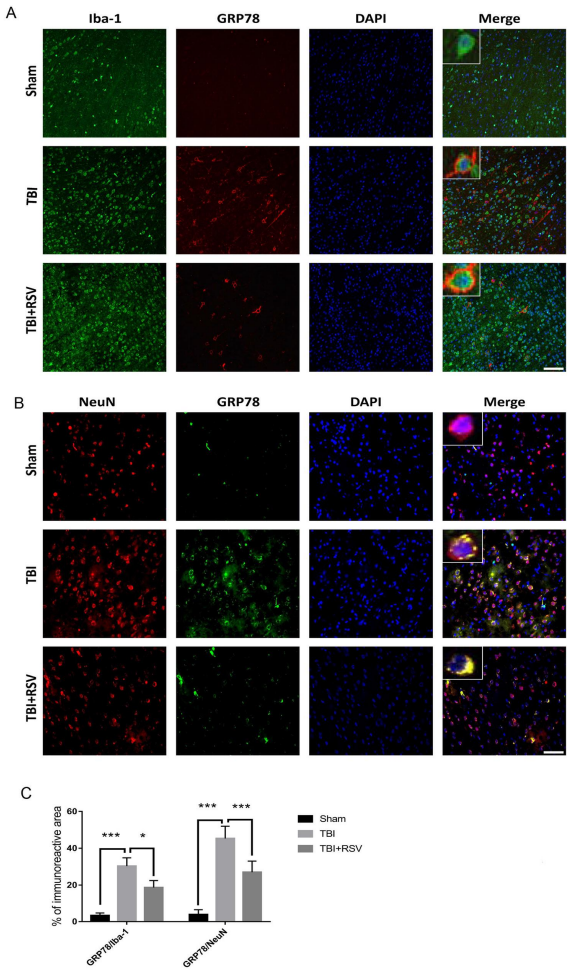

Figure 3. Immunofluorescence staining of mouse brain tissues. A and B show more Iba-1^{+}/GRP78^{+} cells in the cortex ipsilateral to the injury side in the TBI group after 24 h, reversed in the TBI+RSV group. C and D show more NeuN^{+}/GRP78^{+} cells in the TBI group after 24 h, with significant less NeuN^{+}/GRP78^{+} cells in the TBI+RSV group. Scar bar= 50 μm. $^{*}P$<0.05, $^{**}P$<0.01， $^{***}P$<0.001. n = 3 per group. RSV: resveratrol; TBI: traumatic brain injury. one-way ANOVA

3.4 RSV decreases GRP78 immunofluorescence in neurons after TBI

ERS in the cortical neurons was explored by visualizing the NeuN (red) and GRP78 (green) immunofluorescence. TBI increased GRP78 immunofluorescence in neurons in the injured cortex compared to in the sham group, while RSV treatment following TBI significantly decreased GRP78 immunofluorescence (Fig.3B and 3C).

3.5 Cortical IL-1β, and TNF-α levels are reduced by RSV treatment after TBI

To explore whether microglial activation leads to strong neuroinflammation, we assessed the mRNA expression of TNF-α and IL-1β in the injured cortex. We found that IL-1β significantly increased at 24 and 72 h in the TBI group compared to the sham group (Fig.3A and 3B). However, RSV treatment suppressed the elevation of IL-1β compared to the level in the TBI group (Fig.4A and 4B). TNF-α, which is also a regulator of T-cell activation and cause the release of cytokines to induce cell death, exhibited a similar trend at 24 h, but TNF-α expression was not significantly changed at 72 h (Fig.4C and 4D).

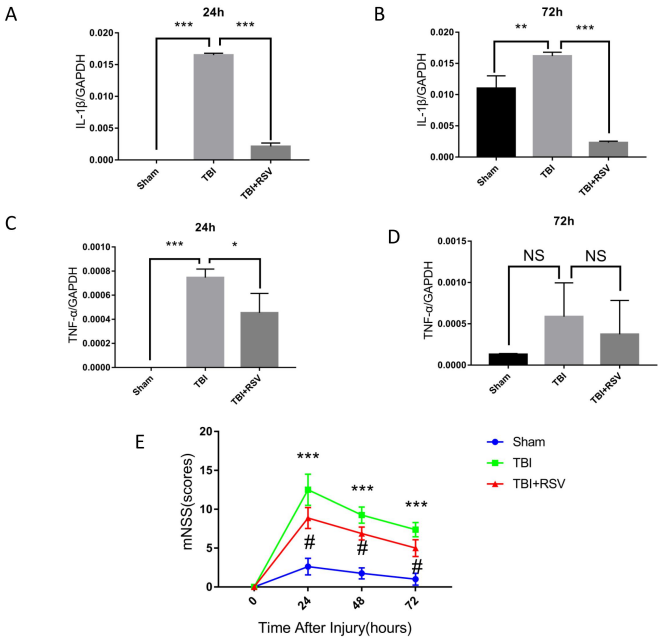

Figure 4. Effect of RSV on IL-1β and TNF-α and neurological dysfunction. The mRNA expressions of IL-1β were highly increased at 24 and 72 h in the TBI group compared to the sham group, but significantly decreased after RSV treatment. TNF-α expression was also obviously elevated at 24 h in the TBI group, but no significant difference after RSV treatment (A-D, n=4, $^{*}P<0.05$, $^{***}P<0.001$). E shows the sham group did not show any neurological dysfunction after surgery. The mNSS score was significantly higher in the TBI group compared to the sham group at 24, 48 and 72 h, $^{***}P<0.001$. RSV improved mNSS at all time points compared it to the TBI group, $^{\#}P<0.001$ (n = 6). NS: nonsignificant difference; RSV: resveratrol; TBI: traumatic brain injury.figure 4A-D: one-way ANOVA,figure 4E:repeated measure two-way ANOVA

3.6 RSV improves neurological behavior after TBI

The sham group did not show any neurological dysfunction after surgery, based on mNSS. Significant functional deficits were exhibited in the TBI group at 24, 48, and 72 h after TBI. The TBI+RSV group showed significant improvements in the neurological function at 24, 48, and 72 h compared to the TBI group (Fig. 4E).

4. Discussion

In this study, the ERS pathway-related mechanisms underlying the suppression of TBI-induced apoptosis and inflammation by RSV treatment were investigated. The main findings were as follows: (1) activation of the ERS signaling pathway occurred in the acute phase of TBI and was inhibited by RSV; (2) apoptosis, which was inhibited by RSV, occurred along with ERS activation; and (3) neurological impairment was attenuated by RSV. These results imply that RSV could be a neuroprotector that hinders TBI-triggered apoptosis via the inhibition of the ERS pathway.

As the objective of this in vivo study was to examine the anti-apoptotic properties of post-TBI RSV treatment (involving the ERS signaling pathway), we adopted a CCI model to perform basic experiments. In a more precise terms, we sought to explore whether RSV suppressed markers of ERS and apoptosis in injured brain. We hypothesized that ERS markers are fundamental regulators or modulators of post-TBI apoptosis, and natural compounds such as RSV may be useful for reducing neuroinflammation and long-term neurodegeneration. In other TBI study, ERS triggered UPR and other adaptive processes [14]. In addition RSV has also been shown to shield astrocytes from the snaring effects of TBI[15].In our experiment, we found that CCI increased GRP78 and CHOP, together with caspase-3 and caspase- 12, indicating a change from the preliminary neuronal repair process to a cell death response.

RSV's ability to modulate and limit ERS and apoptosis via CHOP downregulation has been demonstrated in the brain injury[16]. However, RSV's effects on ERS and apoptosis remain controversial. We attempted to elucidate some of these discrepancies. Using a CCI model, we showed that RSV reduces GRP78 and CHOP levels to directly or indirectly reduce caspase-12- and caspase-3-mediated apoptosis, thereby controlling cellular fate. The data from our research, provide the foundation on which to investigate apoptosis and cellular fate involving ERS. Recently, caspase-12 has been linked to ERS-related pro-apoptotic signals (which are

independent of the extrinsic or intrinsic apoptotic pathways) [17]. Our study has provided an overview of the effects of RSV on these pathways.

IL-1β is one cytokine that has a critical function in the immune response in TBI, as well as the causing increased ROS generation[18]. TNF-α is also the pro-inflammation cytokine, which released at the early stage of TBI. Higher TNF-α levels, secondary to TBI, have been suggested as a critical reason for brain tissue injury responses[19]. Therefore, targeting IL-1β and TNF-α may help to relieve TBI. We can see from our data that TBI increased inflammatory markers that have been confirmed. The inflammatory cytokines (IL-1β and TNF-α) in the brain were increased after TBI in our experiment. Additionally, IL-1β and TNF-α were downregulated by RSV in the CCI model, confirming effect of RSV on microglial activation in vivo.

Immunofluorescence related to the ERS marker GRP78 was elevated in cortical neurons, confirming ERS activation following TBI, which was decreased by RSV treatment. We also showed that GRP78 was increased in Iba-1-positive cells following TBI, indicating that ERS occurs not only in neurons but also in microglia. Additionally, our investigational natural compound, RSV, decreased Iba-1/GRP78 co-labeling in primary microglial cells after TBI. Previous research has indicated that GRP78 is distributed in different regions, such as neurons and microglia, due to ERS[20]. Hence, the increased GRP78 in activated microglia and neurons in the cortex of our TBI mice matches the findings of previous research[20]. Goldstein et al. reported that neurons expressing CHOP tended to be surrounded by activated Iba-1-positive microglial cells, implying that microglial activation is partially a response to ERS in neurons[21].

Clinically, TBI impairs neurological functions, so new approaches to improve TBI outcomes are crucial. When the TBI mice were treated with the ERS inhibitor RSV, markers of cell death and neurological dysfunction were attenuated inaccordance to previous experiment that revealed that RSV improved the behavioral and motor functions of mice that had sustained CCI[22]. We hypothesize that there is a certain window of time for RSV to reduce the detrimental effects of ERS, together with apoptosis, to enhance neurological function after TBI. However, further research is necessary to confirm and further explore the basic mechanism. Extensive research involving a more clinically relevant injury model is required to consolidate the ERS-related anti-apoptotic mechanism and the effects of RSV. Our study is limited because inhibitors of ERS markers were not used to determine whether reverse outcomes occur that are related to the apoptosis cascade. Using inhibitors of ERS markers

would allow more definitive conclusions to be drawn.moreover other key markers in the mechanism of ERS were not investigated;inparticular eukaryotic initiation factor 2 alpha phosphatase which has been shown to reduce tissue damage if inhibited after TBI[23].

In conclusion, we found that RSV treatment can relieve secondary brain injury and protect brain tissue after TBI by affecting ERS, apoptosis, and neuroinflammation. This study shows that RSV may become an important treatment for TBI disability.

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Author contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design was done by Mark Nyanzu, Zhezhe Sun ,Qichuan ZhuGe, and Lijie Huang. Acquisition of data was done by Mark Nyanzu, Zhezhe Sun, and Felix Siaw-Debrah. Analysis and interpretation of data were done by Xiaohong Zhu and Xiao Lin. Drawing of the manuscript was done by Mark Nyanzu, Felix Siaw-Debrah and Ying Zhang. Critical revision of the manuscript for important intellectual content was done by Qichuan ZhuGe and Lijie Huang. Statistical analysis was done by Haoqi Ni, Xiaohong Zhu and Su Yang.

References

[1] Langlois JA, Rutland-Brown W, Wald MM: **The epidemiology and impact of traumatic brain injury: a brief overview**. *J Head Trauma Rehabil* 2006, **21**(5):375-378.

[2] Hernandez-Ontiveros DG, Tajiri N, Acosta S, Giunta B, Tan J, Borlongan CV: **Microglia activation as a biomarker for traumatic brain injury**. *Front Neurol* 2013, **4**:30.

[3] Woodcock T, Morganti-Kossmann MC: **The role of markers of inflammation in traumatic brain injury**. *Front Neurol* 2013, **4**:18.

[4] Turtzo LC, Lescher J, Janes L, Dean DD, Budde MD, Frank JA: **Macrophagic and microglial responses after focal traumatic brain injury in the female rat**. *J Neuroinflammation* 2014, **11**:82.

[5] Liu Y, Kintner DB, Chanana V, Algharabli J, Chen X, Gao Y, Chen J, Ferrazzano P, Olson JK, Sun D: **Activation of microglia depends on Na+/H+ exchange-mediated H+ homeostasis**. *J Neurosci* 2010, **30**(45):15210-15220.

[6] Gao Y, Zhang MY, Wang T, Fan YY, Yu LS, Ye GH, Wang ZF, Gao C, Wang HC, Luo CL *et al*: **IL-33/ST2L Signaling Provides Neuroprotection Through Inhibiting Autophagy, Endoplasmic Reticulum Stress, and Apoptosis in a Mouse Model of Traumatic Brain Injury**. *Front Cell Neurosci* 2018, **12**:95.

[7] Diehl JA, Fuchs SY, Koumenis C: **The cell biology of the unfolded protein response**. *Gastroenterology* 2011, **141**(1):38-41, 41 e31-32.

[8] Hetz C, Chevet E, Harding HP: **Targeting the unfolded protein response in disease**. *Nat Rev Drug Discov* 2013, **12**(9):703-719.

[9] Stadtman ER, Moskovitz J, Berlett BS, Levine RL: **Cyclic oxidation and reduction of protein methionine residues is an important antioxidant mechanism**. *Mol Cell Biochem* 2002, **234-235**(1-2):3-9.

[10] Aronowski J, Zhao X: **Molecular pathophysiology of cerebral hemorrhage: secondary brain injury**. *Stroke* 2011, **42**(6):1781-1786.

[11] Tellone E, Galtieri A, Russo A, Giardina B, Ficarra S: **Resveratrol: A Focus on Several Neurodegenerative Diseases**. *Oxid Med Cell Longev* 2015, **2015**:392169.

[12] Lopez MS, Dempsey RJ, Vemuganti R: **Resveratrol neuroprotection in stroke and traumatic CNS injury**. *Neurochem Int* 2015, **89**:75-82.

[13] Zou P, Liu X, Li G, Wang Y: **Resveratrol pretreatment attenuates traumatic brain injury in rats by suppressing NLRP3 inflammasome activation via SIRT1**. *Mol Med Rep* 2018, **17**(2):3212-3217.

[14] Rubovitch V, Shachar A, Werner H, Pick CG: **Does IGF-1 administration after a mild traumatic brain injury in mice activate the adaptive arm of ER stress?** *Neurochem Int* 2011, **58**(4):443-446.

[15] Lin CJ, Chen TH, Yang LY, Shih CM: **Resveratrol protects astrocytes against traumatic brain injury through inhibiting apoptotic and autophagic cell death**. *Cell Death Dis* 2014, **5**:e1147.

[16] Pan S, Li S, Hu Y, Zhang H, Liu Y, Jiang H, Fang M, Li Z, Xu K, Zhang H *et al*: **Resveratrol post-treatment protects against neonatal brain injury after hypoxia-ischemia**. *Oncotarget* 2016, **7**(48):79247-79261.

[17] Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J: **Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta**. *Nature* 2000, **403**(6765):98-103.

[18] Mortezaee K, Khanlarkhani N, Beyer C, Zendedel A: **Inflammasome: Its role in traumatic brain and spinal cord injury**. *J Cell Physiol* 2018, **233**(7):5160-5169.

[19] Rostworowski M, Balasingam V, Chabot S, Owens T, Yong VW: **Astrogliosis in the neonatal and adult murine brain post-trauma: elevation of inflammatory cytokines and the lack of requirement for endogenous interferon-gamma**. *J Neurosci* 1997, **17**(10):3664-3674.

[20] Fan H, Tang HB, Kang J, Shan L, Song H, Zhu K, Wang J, Ju G, Wang YZ: **Involvement of endoplasmic reticulum stress in the necroptosis of microglia/macrophages after spinal cord injury**. *Neuroscience* 2015, **311**:362-373.

[21] Harvey LD, Yin Y, Attarwala IY, Begum G, Deng J, Yan HQ, Dixon CE, Sun D: **Administration of DHA Reduces Endoplasmic Reticulum Stress-Associated Inflammation and Alters Microglial or Macrophage Activation in Traumatic Brain Injury**. *ASN Neuro* 2015, **7**(6).

[22] Singleton RH, Yan HQ, Fellows-Mayle W, Dixon CE: **Resveratrol attenuates behavioral impairments and reduces cortical and hippocampal loss in a rat controlled cortical impact model of traumatic brain injury**. *J Neurotrauma* 2010, **27**(6):1091-1099.

[23] Hood KN, Zhao J, Redell JB, Hylin MJ, Harris B, Perez A, Moore AN, Dash PK: **Endoplasmic Reticulum Stress Contributes to the Loss of Newborn Hippocampal Neurons after Traumatic Brain Injury**. *J Neurosci* 2018, **38**(9):2372-2384.