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Resveratrol attenuates the $CoCl_2$-induced hypoxia damage by regulation of lysine β-hydroxybutyrylation in PC12 cells

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Abstract

Background Stroke is a cerebrovascular disease that is the main cause of death and disability worldwide. Hypoxia is a major factor that causes neuronal damage and even cellular death. However, the mechanism and therapeutic drugs for hypoxia are not completely understood.

Methods In this study, PC12 cells (a rat adrenal pheochromocytoma cell line) were exposed to Cobalt chloride ($CoCl_2$) to induce hypoxia. Using this cell model, the impacts of hypoxia on cell viability, proliferation, reactive oxygen species (ROS), and the levels of lysine β-hydroxybutyrylation (Kbhb) and the inflammatory signaling factor P65 were examined. In addition, we explored the ability of resveratrol (RES) to alleviate $CoCl_2$-induced hypoxia damage.

Results RES attenuated $CoCl_2$-induced decreases of cell viability and cell proliferation and increase of ROS production in PC12 cells. $CoCl_2$ downregulated Kbhb in PC12 cells, but RES alleviated this effect. In addition, upregulated Kbhb by 3-hydroxybutyric acid sodium could partially recover the $CoCl_2$-induced hypoxia damage to PC12 cells, including cell viability, cell proliferation, oxidative stress, and the protein level of the inflammatory signaling factor P65.

Conclusion Our results indicate that RES protects against $CoCl_2$-induced hypoxia damage in PC12 cells by modulating Kbhb, a novel post-translational modification.

Keywords Resveratrol, $CoCl_2$, Hypoxia, Lysine β-hydroxybutyrylation, PC12 cells, Reactive oxygen species

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Background

Stroke is one of the causes of death and disability worldwide. Due to its rapid onset, many complications and narrow time window, the most effective interventions for the clinical treatment of stroke are endovascular thrombectomy and recombinant tissue plasminogen activator [1]. However, a large proportion of patients are not effectively treated within a short window. The inadequate oxygen (hypoxia) caused by stroke stimulates cells to activate multifaceted cellular responses that lead to cellular death [2, 3]. PC12 cells, a rat pheochromocytoma cell line, have similar properties to cultured neurons in vitro and are widely used in the study of nervous system diseases [4, 5]. Cobalt chloride ($CoCl_2$), a hypoxia mimetic agent, was used to construct a hypoxia model in PC12 cells [6]. However, the exact molecular mechanism and therapeutic drugs involved in stroke pathophysiology are not completely understood.

An increasing number of studies have shown that oxidative stress is one of the main pathophysiological mechanisms of stroke [7]. The overproduction of reactive oxygen species (ROS) is a major cause of oxidative stress and leads to neuronal damage [8]. In addition, as a key regulatory way of cell function, post-translational modifications (PTMs) play pivotal roles in many physiological processes [9, 10]. With the development of mass spectrometry, an increasing number of acylation modification types, such as acetylation, crotonylation, butyrylation, and ubiquitination, have been identified [11–14]. Disturbance of the homeostasis of protein methylation, acetylation and ubiquitination leads to various neurological disorders [15–17]. Lysine β-hydroxybutyrylation (Kbhb) has not been reported in neurological diseases until now. Kbhb is catalyzed by a substrate (β-hydroxybutyric acid) [18]. β-Hydroxybutyric acid (BHBA), an endogenous ketone body, plays a neuroprotective role in many neurodegenerative diseases, such as the inhibition of abnormal neuronal discharge, the inhibition of neuroinflammation and the treatment of depressive disorders [19, 20]. In addition, the regulation of Kbhb and its recovery under hypoxia-induced nervous system injury have not been investigated. The results may provide a potential therapeutic target in stroke or neurodegenerative diseases.

RES (trans-3,5,4'-trihydroxystilbene), a well-known polyphenol compound, is derived from grapes, berries, peanuts, etc [21–23]. With clinical potential, RES has been shown to be an effective treatment for several diseases, including diabetes [24], cancer [25], neurological [26] and cardiovascular diseases [27]. Among these factors, RES plays a neuroprotective role by reducing oxidative damage, cognitive impairment, and mitochondrial dysfunction [28–30]. However, whether RES supplementation protects against nervous injury following hypoxia-$CoCl_2$ exposure has not been reported.

In this study, the neurotoxicity of $CoCl_2$ was evaluated using PC12 cells in vitro. Cell viability, proliferation, ROS production, and the levels of Kbhb and the inflammatory signaling factor P65 were examined. In addition, we utilized a natural antioxidant (RES) to study whether the amelioration of ROS and Kbhb alleviated the toxicity of $CoCl_2$ to PC12 cells. This study may provide novel strategies for neurotoxicity stroke caused by hypoxia and understanding the underlying mechanism involved.

Methods

Reagents and antibodies

Cobalt chloride hexahydrate ($CoCl_2{\cdot}6H_2O$) was obtained from Sigma (C8661, St. Louis, USA). RES was purchased from Yuanye Technology (T94028, Shanghai, China). The antibody against β-hydroxybutyryllysine (1:1000) was obtained from PTM Biolab (PTM-1201RM, Hangzhou, China). The antibody GAPDH (1:6000) was purchased from OriGene (TA802519, Maryland, USA). The P65 antibody (1:2000) was purchased from HUABIO (ET1603-12, Hangzhou, China). The histone 3 antibody (1:800) was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). 3-Hydroxybutyric acid sodium was purchased from MedChemExpress (HY-W010452, New Jersey, USA).

Cell culture

PC12 cells (a rat pheochromocytoma cell line) were obtained from the Cell Bank of the Chinese Academy of Sciences. Then, the PC12 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 5% horse serum and 1% antibiotic mixture and incubated at 37 °C with 5% CO_2 in an incubator. Cells were seeded in 6-well plates or 96-well plates at a density of 1×10^5 cells/well or 5000 cells/well, respectively.

CCK-8 assay

Cell viability was evaluated by a Cell Counting Kit-8 (CCK-8) (C0038, Beyotime Biotechnology, Shanghai, China). PC12 cells were grown and incubated with $CoCl_2$, RES and 3-hydroxybutyric acid sodium (independently or in combination). Cells were treated with $CoCl_2$ (5 μM, 50 μM, or 100 μM) in a 96-well plate for 24, 48, or 72 h to evaluate the cytotoxic effect of the drug; 48 h was used for subsequent experiments. Both RES (50 μM) and 3-hydroxybutyric acid sodium were added to $CoCl_2$ to evaluate its protective effect. Then, 10 μL of CCK-8 solution was added to each well 4 h before the end of the 24-, 48-, or 72-hour incubation. The difference in the absorbance at 450 nm was used to evaluate the possible cytotoxic effects of $CoCl_2$, RES and 3-hydroxybutyric acid sodium. The average of replicates was used for each group, and the results are expressed as a percentage of

cell viability compared to that of untreated cells and are reported as the means and standard deviations (SD).

EdU assay

A 5-ethynyl-2′-deoxyuridine (EdU) cell proliferation detection kit (CX003, Shanghai Epizyme) was used to determine the level of cell proliferation. EdU reagent was added to the cells after treatment for 48 h, and the cells were incubated at 37 °C for 2 h. The cells were washed three times with serum-free medium to remove the uncombined EdU dye. Photographs were taken using a fluorescence microscope (OLYMPUS IX73, Japan). The number of fluorescence-positive cells reflected the level of cell proliferation. EdU positive cells (%) were calculated as the ratio of EdU staining cells to Hoechst staining cells and multiplied by 100 (%) by Image J software.

Intracellular ROS production

Intracellular ROS levels were determined with an ROS detection kit (R6033, US Everbright). Briefly, 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) was added to the PC12 cells after treatment for 48 h and incubated at 37 °C for 30 min. Subsequently, the cells were washed with phosphate buffered saline three times to remove the uncombined ROS dye. The fluorescence was measured using a microplate reader (PerkinElmer EnSpire, USA).

Western blotting

PC12 cells were homogenized in radio immunoprecipitation assay buffer (R0020, Solarbio) supplemented with 1% protein and phosphorylation degradation inhibitors to perform protein extraction. Lysates were centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was collected for protein analysis. The nuclear fraction of PC12 cells was extracted using a nuclear protein extraction kit (EX1470, Solarbio). The samples were denatured in 4× loading buffer, and the proteins were separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gels. Following electrophoresis, the proteins were transferred onto a polyvinylidene fluoride membrane (0.45 μm, Amersham) in transfer buffer at 280 mA for 90 min. The obtained membranes were incubated with 5% nonfat dry milk in 1×Tris buffered saline with Tween-20 (TBST) for 1 h at room temperature and washed 3 times in TBST to remove the milk. Then, the membranes were incubated with primary antibodies to detect β-hydroxybutyryllysine and P65 diluted in TBST overnight at 4 °C. The next day, following 3 washes with TBST, the membranes were incubated with secondary peroxidase-conjugated goat anti-mouse or anti-rabbit antibodies for 1 h at room temperature. After washing 3 times in TBST, the membranes were imaged with a gel imaging system (Healthcare AI600, USA). Analysis of Kbhb was conducted by quantifying the sum gray values of all the detected bands normalized to the bands detected by GAPDH. The p65 in nuclear fractions of PC12 cells was quantified by normalizing the gray values of target bands of p65 to those of histone 3. The gray values of the bands in the membranes were analyzed using Image J software (version 1.51, National Institutes of Health, MD, USA).

Statistical analysis

The data are expressed as the mean ± SD of three independent experiments and were analyzed statistically using One-way ANOVA with GraphPad Prism (version 5.0, USA). Differences ($p < 0.05$) were regarded as significant.

Results

RES protected against $CoCl_2$-induced cytotoxicity to PC12 cells

We treated PC12 cells with different concentrations (5 μM, 50 μM and 100 μM) of $CoCl_2$ for 24, 48, and 72 h to measure cell viability using a CCK-8 assay. Our results showed that there was no significant difference in PC12 cell viability between the 5 μM $CoCl_2$ treatment group and the control group. However, exposure to 50 μM and 100 μM $CoCl_2$ caused cytotoxicity (Fig. 1a and b).

Compared with those in the control group, the morphology of the PC12 cells in the $CoCl_2$-treated group was abnormal (Fig. 2a). Furthermore, we wanted to measure whether RES protected against $CoCl_2$-induced cytotoxicity in PC12 cells in vitro. RES (50 μM) was added to the cells, which were then cultured with $CoCl_2$. Our results showed that compared with $CoCl_2$, RES ameliorated $CoCl_2$-induced changes in cell viability and morphology (Fig. 2a and b). These results indicated that RES significantly protected against $CoCl_2$-induced changes in PC12 cell viability and morphology.

RES protected against $CoCl_2$-induced proliferation of PC12 cells

EdU was used to assess the proliferation of cells, and EdU-positive cells indicated PC12 cell proliferation. PC12 cells were exposed to 100 μM $CoCl_2$ combined with 50 μM RES for 48 h. Compared with $CoCl_2$ treatment, RES treatment restored the proliferation of $CoCl_2$-treated PC12 cells. The results indicated that RES significantly protected against $CoCl_2$-induced PC12 cell proliferation (Fig. 3).

RES inhibited $CoCl_2$-induced ROS production in PC12 cells

In this study, we evaluated whether RES protected against $CoCl_2$-induced ROS production in PC12 cells by mimicking hypoxia. We examined the ROS levels in PC12 cells exposed to $CoCl_2$ combined with RES. Our results showed that $CoCl_2$ significantly induced ROS production

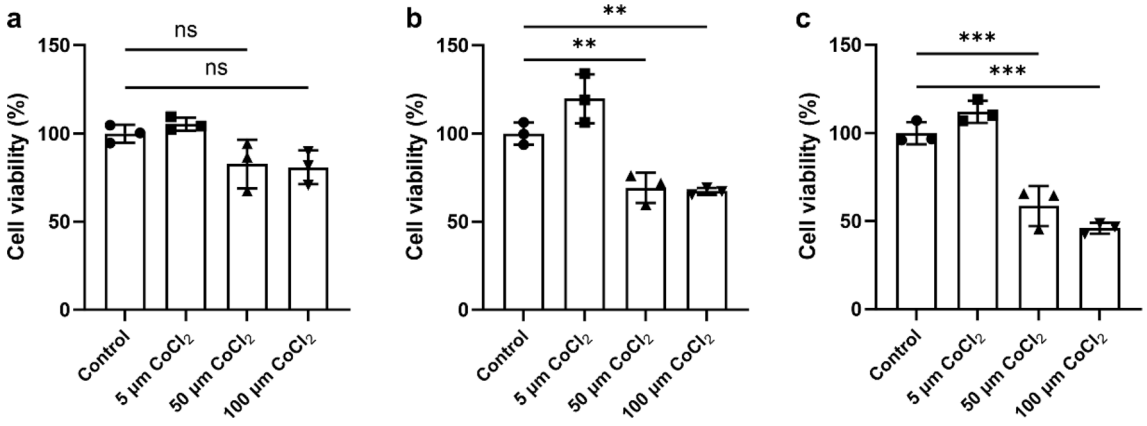

Fig. 1 Effect of $CoCl_2$ on the viability of PC12 cells. (**a**-**c**) PC12 cells were exposed to 5 μM, 50 μM, or 100 μM $CoCl_2$ for assessment of cell viability at 24 h (**a**), 48 h (**b**) and 72 h (**c**) using the CCK-8 assay. $n = 3$. The data conformed to normal distribution analyzed by Shapiro-Wilk test. The data are presented as the mean ± SD. Differences between groups were assessed by one-way ANOVA. $^{**}p < 0.01$ and $^{***}p < 0.001$

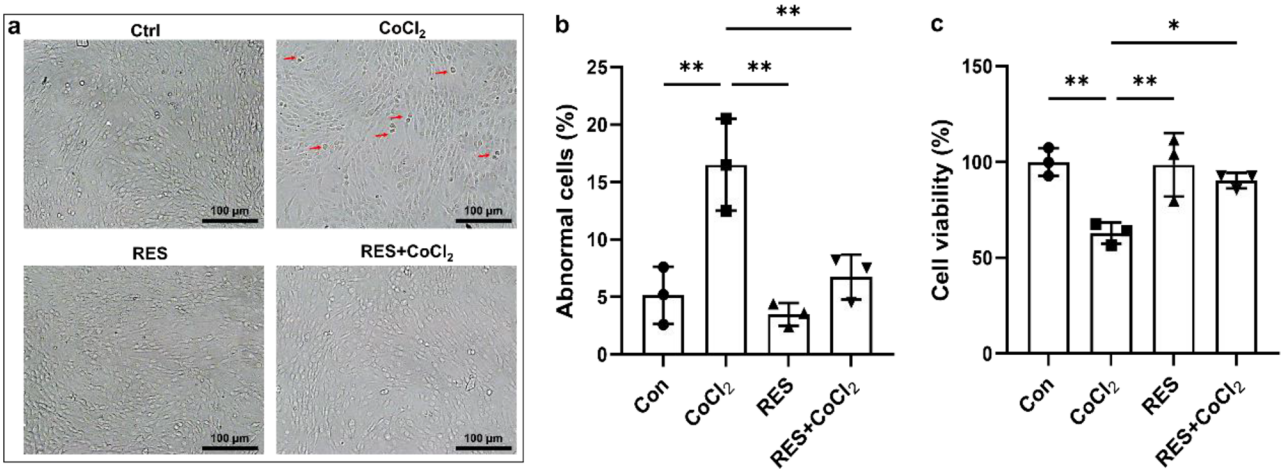

Fig. 2 Effect of $CoCl_2$ combined with resveratrol (RES) on the viability of PC12 cells. PC12 cells were exposed to 100 μM $CoCl_2$, 50 μm RES, and 100 μM $CoCl_2$ plus 50 μm RES for 48 h. (**a**) Morphological characterization of PC12 cells was performed using an optical microscope (the red arrows for abnormal morphology); scale bars in the figures indicate 100 μm. (**b**) The percentage of abnormal cells in different groups have been quantified. (**c**) Cell viability was examined by CCK-8 assay. The data are presented as the mean ± SD. $n = 3$. The data conformed to normal distribution analyzed by Shapiro-Wilk test. Differences between groups were assessed by one-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$

in PC12 cells, while RES could inhibit the increase of ROS production induced by $CoCl_2$ (Fig. 4).

RES attenuated $CoCl_2$-induced decreases in the level of Kbhb

PC12 cells were exposed to $CoCl_2$ (5, 50 or 100 μM), and the level of Kbhb was measured. Our results showed that 5-100 μM $CoCl_2$ decreased the level of Kbhb in PC12 cells (Fig. 5a). Furthermore, we examined whether RES alleviated the $CoCl_2$-induced decrease in the level of Kbhb in PC12 cells. The results showed that RES recovered the $CoCl_2$-induced decrease in the level of Kbhb (Fig. 5b). These results indicated that Kbhb may be involved in $CoCl_2$-induced cytotoxicity in PC12 cells. RES probably protected against $CoCl_2$-induced cytotoxicity by regulating the level of Kbhb.

3-Hydroxybutyric acid sodium protected against $CoCl_2$-induced cytotoxicity to PC12 cells

In this study, we found that $CoCl_2$ decreased the level of Kbhb in PC12 cells and RES can recover the $CoCl_2$-induced decrease in the level of Kbhb (Fig. 5). Since Kbhb is derived from 3-hydroxybutyrate, 3-hydroxybutyric acid sodium can be used to increase Kbhb in cells. Therefore, in this study, we measured whether 3-hydroxybutyric acid sodium can increase the level of Kbhb and protected against $CoCl_2$-induced cytotoxicity to PC12 cells. PC12 cells were exposed to 100 μM $CoCl_2$, 20 mM 3-hydroxybutyric acid sodium, and 20 mM 3-hydroxybutyric acid sodium plus 100 μM $CoCl_2$ for 48 h, and cell viability was examined via a CCK-8 assay. In addition, the level of Kbhb in PC12 cells was assessed by western blotting. The results showed that 3-hydroxybutyric acid sodium

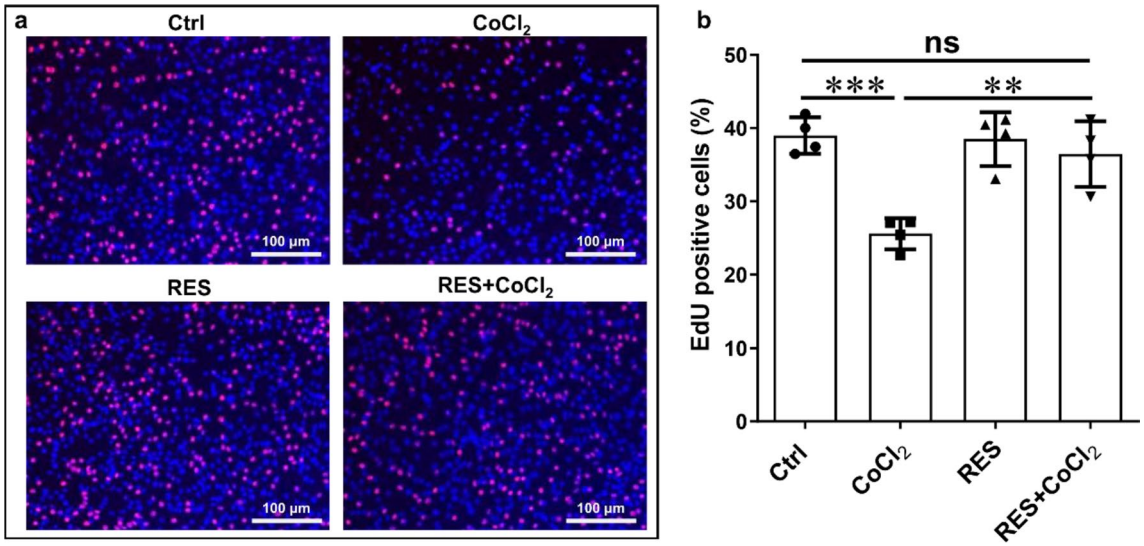

Fig. 3 Effect of $CoCl_2$ combined with RES on PC12 cell proliferation. (**a**) EdU staining (red) and Hoechst staining (blue) of PC12 cells treated with 100 μM $CoCl_2$ combined with 50 μM RES. Scale bar: 100 μm. (**b**) Statistical result of $CoCl_2$ combined with RES on PC12 cell proliferation. EdU positive cells (%) were calculated as the ratio of EdU staining cells to Hoechst staining cells and multiplied by 100 (%). The data are presented as the mean ± SD. $n = 4$. The data conformed to normal distribution analyzed by Shapiro-Wilk test. Differences between groups were assessed by one-way ANOVA. ns: not significant, $^{**}p < 0.01$, $^{***}p < 0.001$

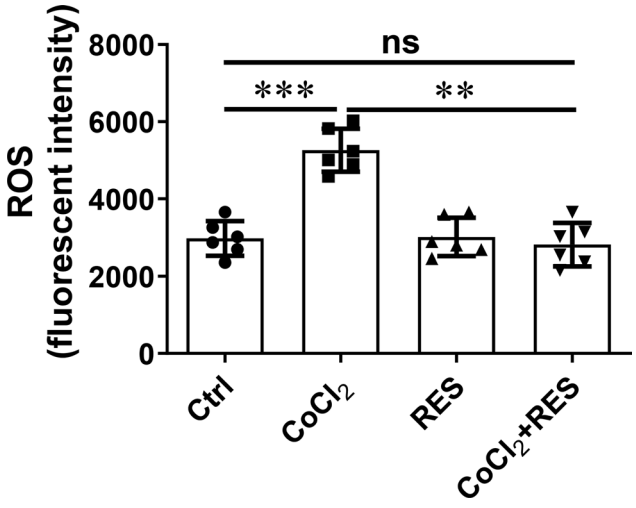

Fig. 4 Effect of RES on $CoCl_2$-induced reactive oxygen species (ROS) production in PC12 cells. PC12 cells were exposed to 100 μM $CoCl_2$, 50 μM RES, and 100 μM $CoCl_2$ plus 50 μM RES for 48 h. ROS production was measured with the ROS fluorescent dye DCFH-DA. The data are presented as the mean ± SD. $n = 6$. The data conformed to normal distribution analyzed by Shapiro-Wilk test. Differences between groups were assessed by one-way ANOVA. ns: not significant, $^{**}p < 0.01$, $^{***}p < 0.001$

increased the level of Kbhb in PC12 cells and recovered the decreases of Kbhb level induced by $CoCl_2$ (Fig. 6a and b) and restored $CoCl_2$-induced cell viability (Fig. 6c).

3-Hydroxybutyric acid sodium protects against $CoCl_2$-induced proliferation of PC12 cells

Since 3-Hydroxybutyric acid sodium recovered the $CoCl_2$-induced decrease of cell viability in PC12 cells (Fig. 6), we examined the intervention effect of 3-Hydroxybutyric acid sodium on $CoCl_2$-induced proliferation of PC12 cells. The results showed that 3-hydroxybutyric acid sodium could rescue the proliferation of $CoCl_2$-treated PC12 cells. The results indicated that 3-hydroxybutyric acid sodium significantly protected against $CoCl_2$-induced PC12 cell proliferation (Fig. 7).

3-Hydroxybutyric acid sodium attenuated $CoCl_2$-induced decreases in protein level of P65

The present study showed that 3-Hydroxybutyric acid sodium significantly protected against $CoCl_2$-induced PC12 cell viability and proliferation, as shown in Figs. 6 and 7. Therefore, we examined whether 3-hydroxybutyric acid sodium could attenuate $CoCl_2$-induced decreases in protein level of P65, which is an important part of the nuclear factor kappa-B (NF-κB) signaling pathway and involved in the inflammatory response. The results showed that 3-hydroxybutyric acid sodium attenuated the $CoCl_2$-induced decrease in the protein level of P65 (Fig. 8).

Discussion

Stroke is an acute cerebrovascular disease that usually results in damage to neurons [31]. The availability of oxygen is important for cellular metabolism. Neurons consume large amounts of energy dependent on oxygen consumption and thus brain oxygen is essential for maintaining neuronal functions. Hypoxia is a major factor that causes neuronal damage in cerebrovascular disease [32]. Studies have reported that overproduction of ROS

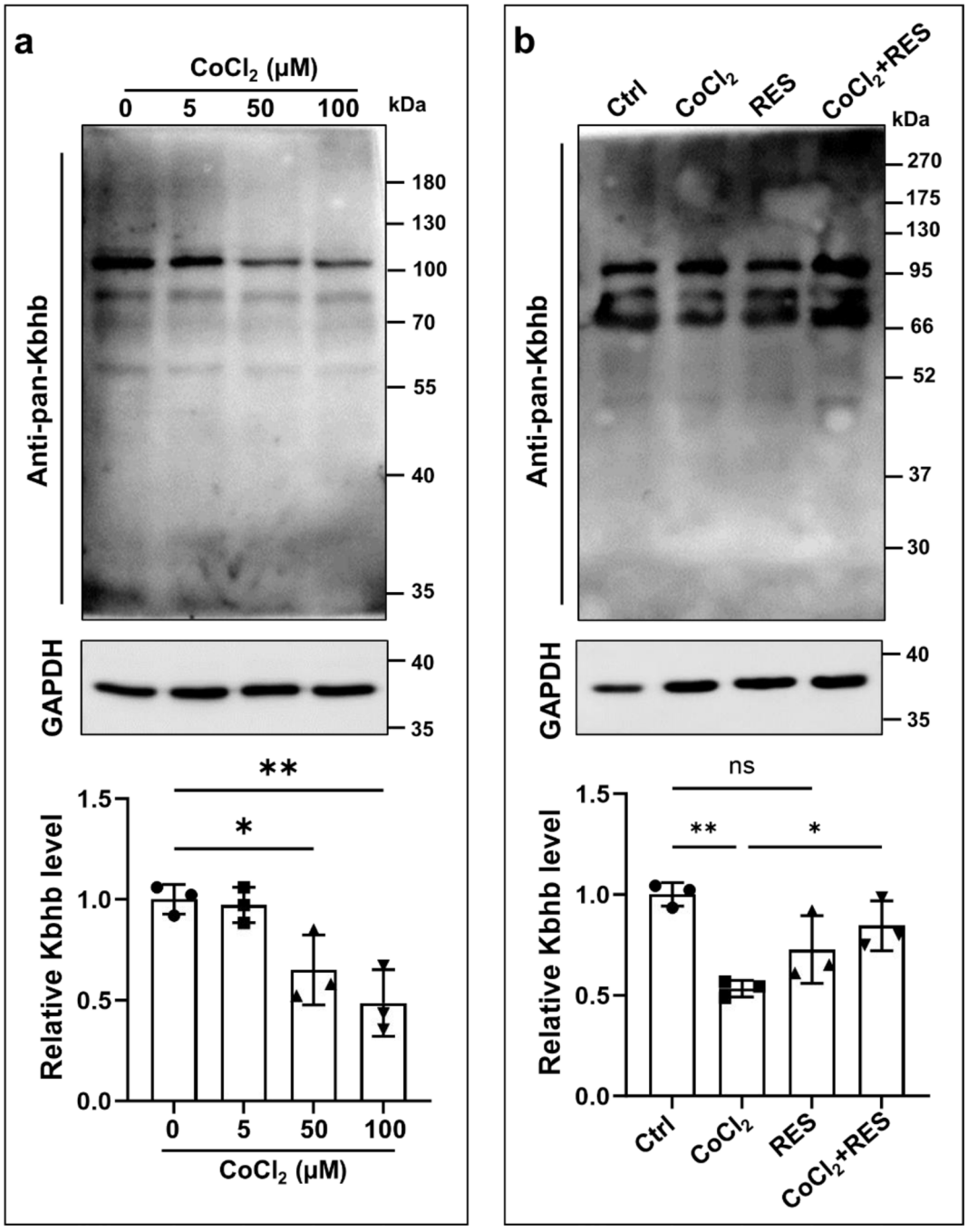

Fig. 5 Effect of $CoCl_2$, RES, and $CoCl_2$ plus RES on the level of Kbhb in PC12 cells. (**a**) PC12 cells were exposed to $CoCl_2$ (5, 50 and 100 μM) for 48 h, and the level of Kbhb was analyzed via western blotting. (**b**) PC12 cells were exposed to 50 μM RES, 100 μM $CoCl_2$, and 50 μM RES plus 100 μM $CoCl_2$ for 48 h, and the level of Kbhb was analyzed via western blotting. The data are presented as the mean ± SD. $n = 3$. The data conformed to normal distribution analyzed by Shapiro-Wilk test. Differences between groups were assessed by one-way ANOVA. *$p < 0.05$, **$p < 0.01$

can trigger oxidative stress to destroy neurons during the pathophysiology of stroke [33, 34]. RES protects PC12 cells from hypoxia (oxygen–glucose deprivation)-induced cell death through its antioxidant effects [35]. In addition, RES improves oxidative stress via Nrf2 signaling pathway [36]. Although the hypoxia model used in this study is different from that used in our study, our findings also show that treatment with RES can effectively suppress hypoxia-induced ROS overproduction in PC12 cells to attenuate cellular oxidative stress.

PTMs increase protein diversity and are closely associated with microenvironment hypoxia. Treatment with $CoCl_2$ reduced the acetylation of histones H3 and H4. It has been also reported that acetylation is involved in

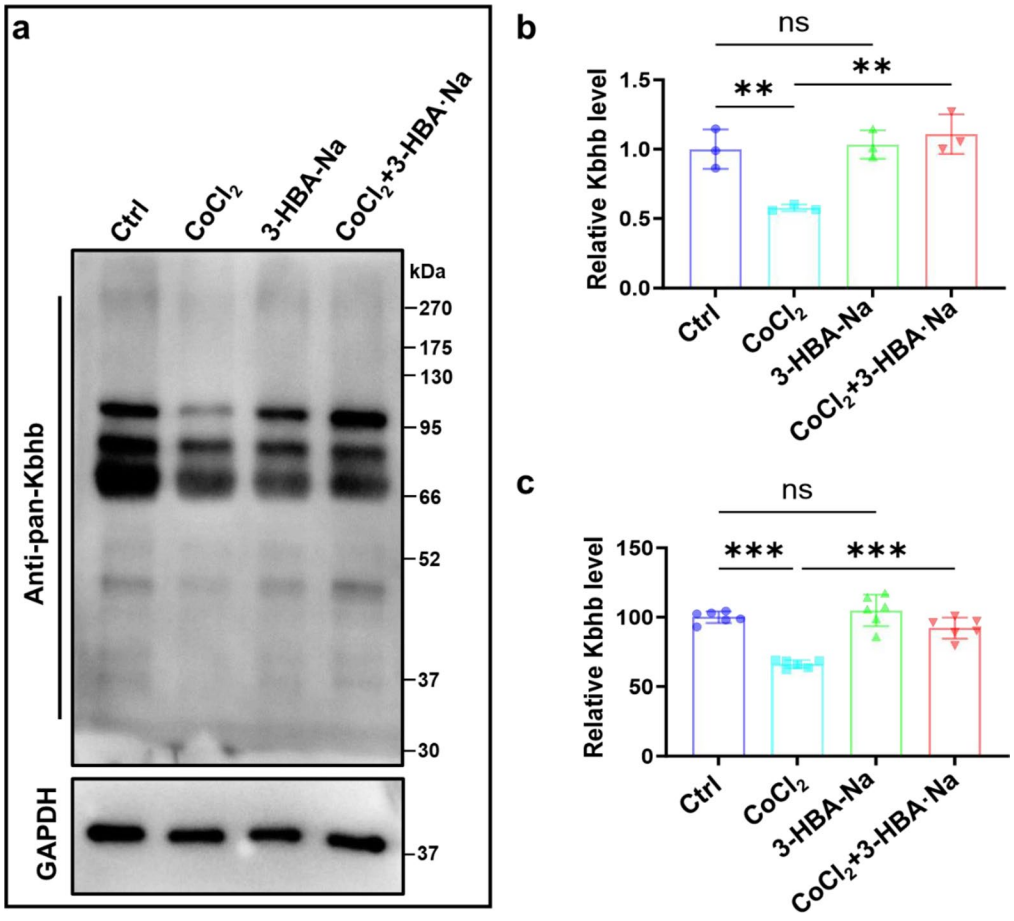

Fig. 6 Effect of 3-hydroxybutyric acid sodium (3-HBA·Na) on the level of Kbhb and viability of PC12 cells. PC12 cells were exposed to 100 μM $CoCl_2$, 20 mM 3-HBA·Na, and 20 mM 3-HBA·Na plus 100 μM $CoCl_2$ for 48 h. (**a** and **b**) The level of Kbhb was examined by western blotting. (**c**) Cell viability was examined via a CCK-8 assay. The data are presented as the mean ± SD. $n = 3$ or 6. The data conformed to normal distribution analyzed by Shapiro-Wilk test. Differences between groups were assessed by one-way ANOVA. $^{**}p < 0.01$, $^{***}p < 0.001$

the fusion of lysosomes with autophagosomes in neurons after ischemic stroke [37]. Protein ubiquitination and SUMOylation increase under hypoxia and are also considered to be neuroprotective. The ubiquitin proteasome system is linked to several signaling pathways that cause injuries after stroke [38–40]. In our study, the level of Kbhb decreased under hypoxia and neuronal injury. These findings suggest that there are differences in the expression level of each protein modification under hypoxia, while an increase in protein modification may have a neuroprotective effect. The occurrence of Kbhb is promoted by the substrate β-hydroxybutyric acid. β-Hydroxybutyric acid, an important energy material, provides energy for the body when it is short of energy through catabolism. A shortage of oxygen and other nutrients provided by the local blood circulation cannot meet the metabolic requirements of neurons, which is the final cause of stroke. Therefore, a decrease in β-hydroxybutyric acid may increase the risk of stroke.

In this context, the effect of RES on the level of Kbhb was examined in PC12 cells exposed to hypoxic injury. RES significantly ameliorated the decrease in the level of Kbhb, demonstrating that the neuroprotective effect of RES relies on an increase in the level of Kbhb. It has been reported that RES enhanced the phosphorylation of p300 and activated the p300, which is the writer of Kbhb [41]. These results suggested that RES ameliorated the $CoCl_2$-indcued decrease in the level of Kbhb by activation of P300. Treatment with 3-hydroxybutyric acid sodium also reversed the decrease in the level of Kbhb induced by $CoCl_2$, demonstrating that 3-hydroxybutyric acid sodium is effective at protecting PC12 cells from hypoxic injury. Therefore, we infer that RES alleviates hypoxia-induced neuronal injury partially via the upregulation of the level of Kbhb. In addition, the protein level of P65 (a member of the NF-κB inflammatory signaling pathway) was examined after treatment with 3-hydroxybutyric acid sodium combined with $CoCl_2$. 3-Hydroxybutyric acid sodium increases $CoCl_2$-induced decreases in the expression of P65, which indicates that neuroprotection of 3-Hydroxybutyric acid sodium increases the level of Kbhb by inhibition of inflammation. In 2003, the Food and Drug

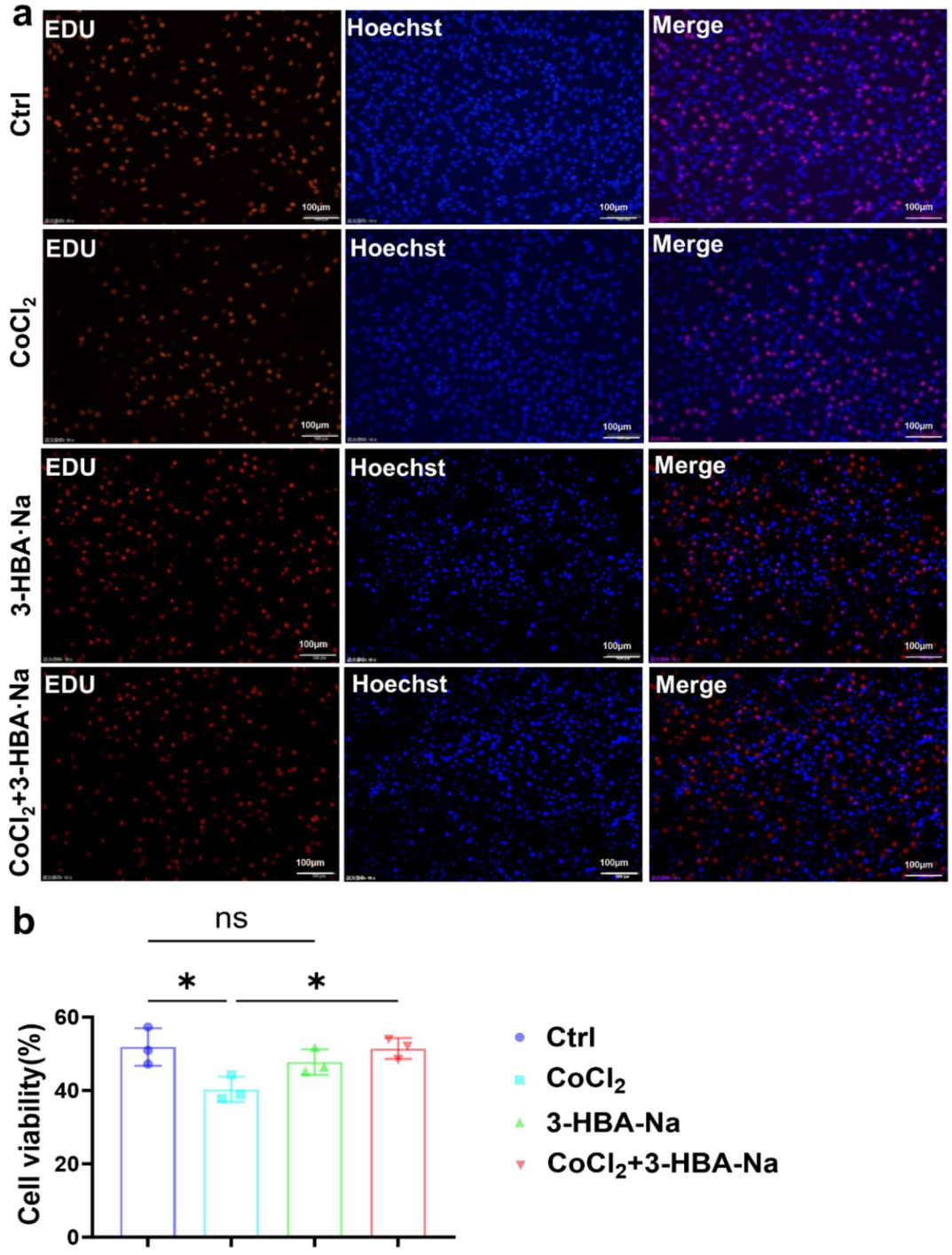

Fig. 7 Effect of 3-hydroxybutyric acid sodium (3-HBA·Na) on the proliferation of PC12 cells. EdU staining (red) and Hoechst staining (blue) of PC12 cells treated with 100 μM $CoCl_2$, 20 mM 3-HBA·Na, and 20 mM 3-HBA·Na plus 100 μM $CoCl_2$. Scale bar: 100 μm. (**b**) Statistical result of 3-HBA·Na combined with RES on PC12 cell proliferation. The data are presented as the mean ± SD. $n = 3$. The data conformed to normal distribution analyzed by Shapiro-Wilk test. Differences between groups were assessed by one-way ANOVA. ns: not significant, $^*p < 0.05$

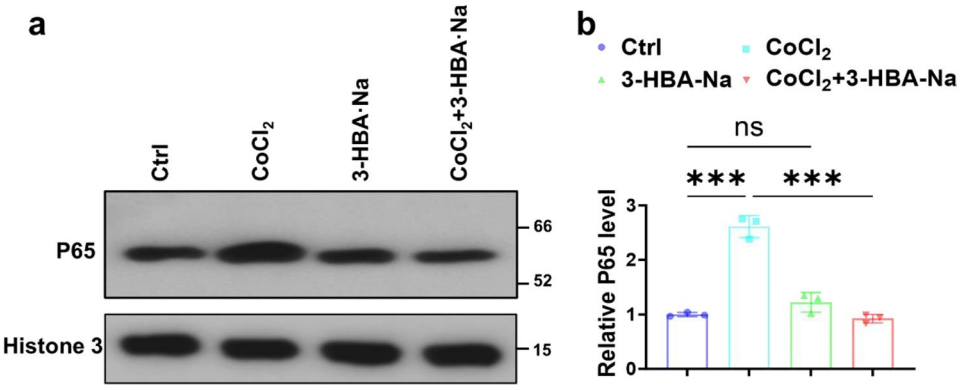

Fig. 8 Effect of 3-hydroxybutyric acid sodium (3-HBA·Na) on the expression of P65. PC12 cells were exposed to 100 μM $CoCl_2$, 20 mM 3-HBA·Na, and 20 mM 3-HBA·Na plus 100 μM $CoCl_2$ for 48 h. The protein level of P65 in the nuclear fraction was analyzed via western blotting (**a** and **b**)

Administration approved bortezomib, which targets the ubiquitin–proteasome system, as an anticancer drug [42]. The implications of this approved RES and targeting Kbhb may include the use of a collection of neuroprotective drugs. However, this study was conducted in vitro model. Future research is needed to explore RES's impact on Kbhb under different hypoxic conditions and possibly in vivo models of ischemic.

Conclusion

In summary, our data demonstrated that RES confers neuroprotection against hypoxia injury by inhibiting the generation of ROS, increasing the level of Kbhb and reducing inflammation. This molecule may become a new target for the clinical treatment of stroke, providing new ideas for the diagnosis and treatment of stroke.

Abbreviations

BHBA	β-Hydroxybutyric acid
CCK-8	Cell Counting Kit-8
$CoCl_2$	Cobalt chloride
DCFH-DA	2′,7′-dichlorodihydrofluorescein diacetate
EdU	5-ethynyl-2′-deoxyuridine
Kbhb	lysine β-hydroxybutyrylation
NF-κB	Nuclear factor kappa-B
PTMs	Post-translational modifications
RES	Resveratrol
ROS	Reactive oxygen species
SD	Standard deviation
TBST	Tris buffered saline with Tween-20

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04171-y.

Supplementary Material 1

Author contributions

YMW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing– original draft; JZ: Formal analysis, Investigation, Methodology, Writing– original draft, Funding acquisition; LS: Formal analysis, Investigation, Methodology, Writing– original draft; DDX: Investigation, Methodology, Writing– review & editing; XMW: Investigation, Methodology, Writing– review & editing; JL: Investigation, Methodology, Writing– review & editing; ZHM: Investigation, Methodology, Writing– review & editing; NX: Investigation, Methodology, Writing– review & editing; QQZ: Conceptualization, Investigation, Methodology, Writing– review & editing; QH: Conceptualization, Investigation, Methodology, Writing– review & editing; YY: Conceptualization, Investigation, Methodology, Writing– review & editing; JGZ: Conceptualization, Investigation, Methodology, Writing– review & editing.

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Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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