

# Protective effect of *Houttuynia cordata* extract on propofol-induced injury of rat hippocampal neurons by regulating PI3K/Akt and Toll-like receptor 4/NF- $\kappa$ B signaling pathway

Hao Fang<sup>a\*</sup>, Zhen Yang<sup>b\*</sup> and Liu Yang<sup>c</sup>

**Objective** This study was to detect the protective effects of *Houttuynia cordata* extract on the damage induced by propofol in hippocampal neuron of rats.

**Methods** Propofol-induced neuron injury model and *H. cordata* extract administration were conducted. Immunofluorescence and immunoblot were conducted for the effect of *H. cordata* extract on neuronal activity and inflammation were detected in this model.

**Results** *H. cordata* extracts increased neuronal activity, and reduced propofol-induced neuronal inflammation levels. *H. cordata* extract also reduced propofol-induced neuronal apoptosis. Mechanically, we noticed *H. cordata* extract activated phosphoinositide 3-kinase/AKT pathway and suppressed Toll-like receptor 4/nuclear factor kappaB pathway, therefore protected propofol-induced injury of rat hippocampal neurons.

**Conclusion** Our findings provide references for anesthetic use in infants and young children. *NeuroReport* 32: 577–582 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

*NeuroReport* 2021, 32:577–582

**Keywords:** *Houttuynia cordata* extract, hippocampal neuron, propofol, PI3K/AKT pathway, Toll-like receptor 4/NF- $\kappa$ B pathway

<sup>a</sup>Department of Anesthesiology, Wuhan Children's Hospital, <sup>b</sup>Department of Anesthesiology, Wuhan JinYinTan Hospital, Wuhan City, Hubei Province and <sup>c</sup>Department of Anesthesiology, Bishan Hospital, Chongqing, China

Correspondence to Liu Yang, MD, Department of Anesthesiology, Bishan Hospital, No. 9 Shuangxing Avenue, Biquan Street, Bishan District, Chongqing City 402760, China  
Tel: +86 023 41411672; e-mail: yangliu\_1223@163.com

\*Hao Fang and Zhen Yang contributed equally to the writing of this article.

Received 25 December 2020 Accepted 29 January 2021

## Introduction

General anesthesia refers to the temporary suppression of the central nervous system when anesthetics are inhaled through the respiratory tract, injected intravenously or intramuscularly into the body [1]. Studies have shown that general anesthesia affects brain development in young animals, and the potential neurotoxicity of anesthetics on infants and young children has aroused widespread concern [2]. Propofol, an intravenous general anesthetic, is a commonly used sedative for induction, maintenance and severe anesthesia in children [3]. Notably, there is growing evidence that propofol induces developmental neurotoxicity that can impair brain development in newborn rats and extend into adulthood [4]. Therefore, it is necessary to understand the mechanism of propofol inducing neuronal developmental toxicity in pediatric anesthesia, so as to provide ideas for developing prevention and treatment strategies.

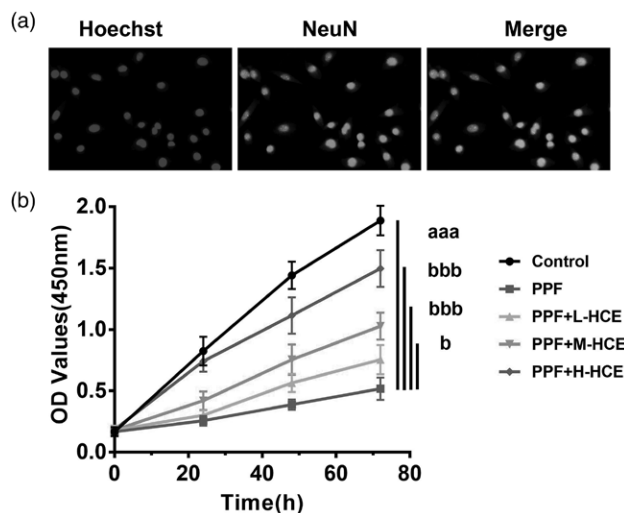
*Houttuynia cordata* is a perennial herb that grows wild in moist, cool places in Asian countries, including China, and has a wide range of biological activities, such as anti-inflammatory, anticancer, antioxidant and immunomodulatory [5,6]. *H. cordata* induces the expression of brain derived neurotrophic factor, phosphorylated cAMP

(Adenosine 3'5' Cyclic Monophosphate)-Response Element Binding protein and phosphorylated AKT Serine/Threonine Kinase 1, has antidepressant effects in mice, and is used in the treatment of mast cell-derived allergic inflammatory diseases by inhibiting the nuclear translocation of nuclear factor kappaB (NF- $\kappa$ B) in HMC-1 cells and preventing the phosphorylation of inhibitor of  $\kappa$ B alpha [7,8]. Interestingly, *H. cordata* can reverse the Th17/Treg balance by regulating the phosphoinositide 3-kinase (PI3K)/Akt/mammalian/mechanistic target of rapamycin signaling pathway, and alleviate the neuropathic pain induced by oxaliplatin in rats [9]. We, therefore, hypothesized that *H. cordata* may affect propofol-induced hippocampal neuron apoptosis and inflammation in rats.

The PI3K/AKT pathway is closely related to neuronal survival because many neurotrophic factors play a protective role in the brain by activating the pathway, thus inhibiting apoptosis [9,10]. Dex can reduce propofol-mediated neuronal apoptosis by enhancing the PI3K/Akt signaling pathway, and reduce the long-term neurotoxicity of propofol on rat brain development [11]. Previous studies showed circRNA00137 inhibited propofol-induced neurotoxicity and neuroinflammation through the PI3K/Akt signaling pathway and played a role in rat brain and nerve cells [12]. Therefore, activation of PI3K/Akt signaling pathway plays an important role in inhibiting propofol-induced neuronal cell injury. This study was objected to investigate the protective effect of *H.*

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Fig. 1



*Houttuynia cordata* extract elevates cell viability of primary hippocampal neurons. (a) Immunofluorescence of NeuN in rat primary neurons. (b) CCK-8 assay revealed cell viability in control, propofol (100  $\mu$ mol/L), propofol + *H. cordata* extract (25  $\mu$ g/mL), propofol + *H. cordata* extract (50  $\mu$ g/mL), propofol + *H. cordata* extract (100  $\mu$ g/mL) groups. Three independent experiments were performed. Data were presented as means  $\pm$  SD. <sup>aaa</sup> $P < 0.001$  versus control; (b)  $P < 0.05$ , <sup>bbb</sup> $P < 0.001$  versus propofol. CCK-8, Cell Counting Kit-8; HCE, *Houttuynia cordata* extract; PPF, propofol.

*cordata* extract on the damage induced by propofol in hippocampal neurons of rats, and to explore the possible mechanisms of PI3K/AKT pathways, so as to provide references for anesthetic use in infants and young children.

## Materials and methods

### Isolation of primary hippocampal neurons

Hippocampal neurons were isolated from Wistar rat embryos on embryonic day 18 utilized for primary neurons isolation. Briefly, about 20 hippocampi tissues were excised, dissected. The attached meninges were removed carefully. Then, the hippocampus was rinsed with PBS. Digest the hippocampus with a mixture of papain (0.5 units/g, Wako, Richmond, Virginia, USA) and deoxyribonuclease I (DNase I) and maintained the mixture at 32°C for 12 min. After digestion, pipetted the mixture with a glass Pasteur pipette several times and filtered the digested solution through a cell strainer (40- $\mu$ m mesh, BD Biosciences, San Jose, California, USA) to remove debris. To inactivate the enzymes, the solution was shaken gently and centrifuged for 10 min at 180g. The centrifuged supernatants were discarded and resuspended the pellet at a density of  $3 \times 10^4$  cells/mL. Subsequently, the cell was seeded on 12-well plates. Rat embryonic hippocampal neurons were cultured in Neurobasal medium (Gibco, Grand Island, Nebraska, USA) containing 2% B27 supplement (50 $\times$ , Invitrogen, Carlsbad, California, USA) and 0.5 mM L-glutamine (Wako) at 37°C in a humidified

atmosphere with 5% CO<sub>2</sub>/95% air. The *H. cordata* extract or propofol was resolubilized in PBS before use and then added directly into the media. Cells were divided into control, propofol (100  $\mu$ mol/L), propofol + *H. cordata* extract (25  $\mu$ g/mL), propofol + *H. cordata* extract (50  $\mu$ g/mL) and propofol + *H. cordata* extract (100  $\mu$ g/mL) groups. Cells were treated with the indicated drugs for 24 h.

### Immunofluorescence

Primary hippocampal neurons were fixed with 4% paraformaldehyde for 10 min and then permeated with PBST (0.1 % Triton X-100 in PBS) followed by blocking with 2% BSA in PBST for 1 h. Then, cells were incubated with primary antibodies against NeuN (1:500; Santa Cruz, Dallas, Texas, USA). Then, fluorescein (FITC)-conjugated secondary antibody (Molecular Probes, Tokyo, Japan) was used at room temperature for 1 h. After washing with PBS, cells were imaged using Leica microscope (Leica, Vista, California, USA, CTR 5000).

### Cell Counting Kit-8 assay

Cells at a density of 1000 cells were seeded into 96-well plates and cultured for 48 h upon the indicated treatment. Then, Cell Counting Kit-8 (CCK-8) was added to the wells for treatment of about 3 h. Then, the absorbance value in different groups was assessed at 490 nm wavelength.

### ELISA

Cell lysates were obtained for tumor necrosis factor-alpha (TNF- $\alpha$ ), (interleukin-6) IL-6 and IL-1 $\beta$  concentration measurement, in accordance with manufacturer's instructions (Dakewe, Beijing, China). Cells were treated with the indicated drugs for 24 h, and then the ELISA assays were performed for assessing the proinflammatory marker levels. Briefly, samples were added into detection wells. Then, removed extra unbound solutions, biotin-conjugated specific antibodies were pipetted into each well followed by the addition of avidin conjugated horseradish peroxidase. After removal of any unbound avidin-enzyme reagent, substrate of the enzyme was added to the wells for color reaction. Stopped the reaction with a correspondent buffer and measured the intensity of the color.

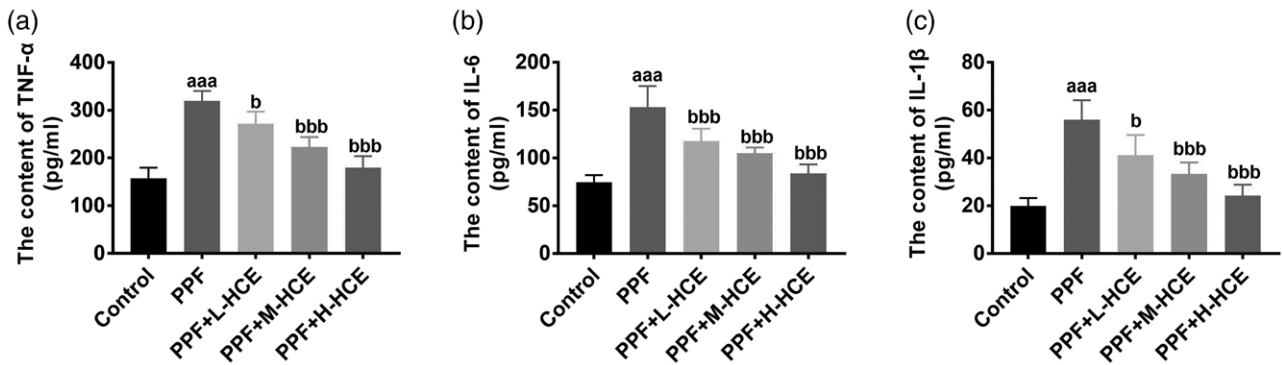
### Flow cytometry

Cells were treated with the indicated drugs for 24 h, and then the flow cytometry assays were performed. For cell apoptosis detection, cells were fixed using 70% alcohol for 24 h at -20°C and stained with FITC and propidium iodide at 37°C for 30 min. Then, cells were detected with a fluorescence-activated cell sorting Calibur flow cytometer. The number of cells in each phase was measured and recorded.

### Immunoblot analysis

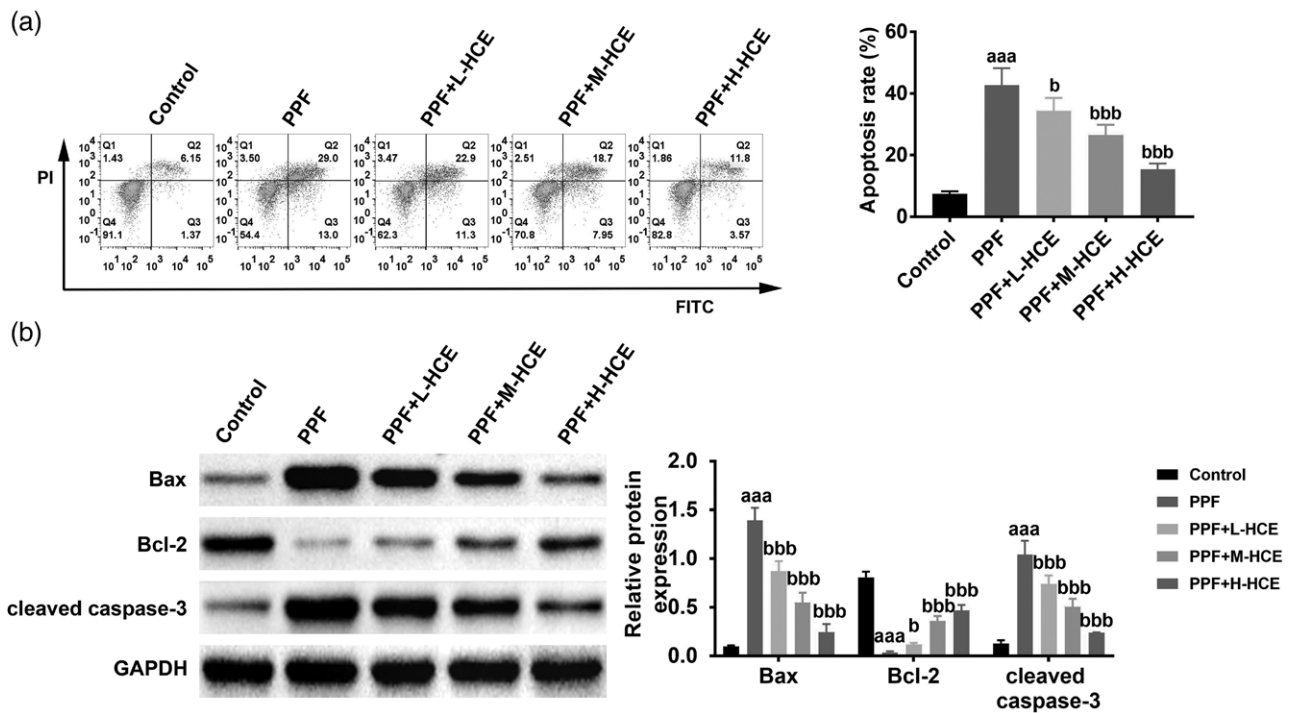
Cells were treated with the drugs for 24 h and lysed using radioimmunoprecipitation buffer (Beyotime, Shanghai, China) and with protein concentration detected with BCA

Fig. 2



*Houttuynia cordata* extract reduces neuron cell inflammation induced by propofol. (a, b, c) The concentration of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  in neuron in control, propofol (100  $\mu$ mol/L), propofol + *H. cordata* extract (25  $\mu$ g/mL), propofol + *H. cordata* extract (50  $\mu$ g/mL), propofol + *H. cordata* extract (100  $\mu$ g/mL) groups assessed by ELISA assay. Three independent experiments were performed. <sup>aaa</sup> $P < 0.001$  versus control; <sup>b</sup> $P < 0.05$ , <sup>bbb</sup> $P < 0.001$  versus propofol. HCE, *Houttuynia cordata* extract; PPF, propofol.

Fig. 3

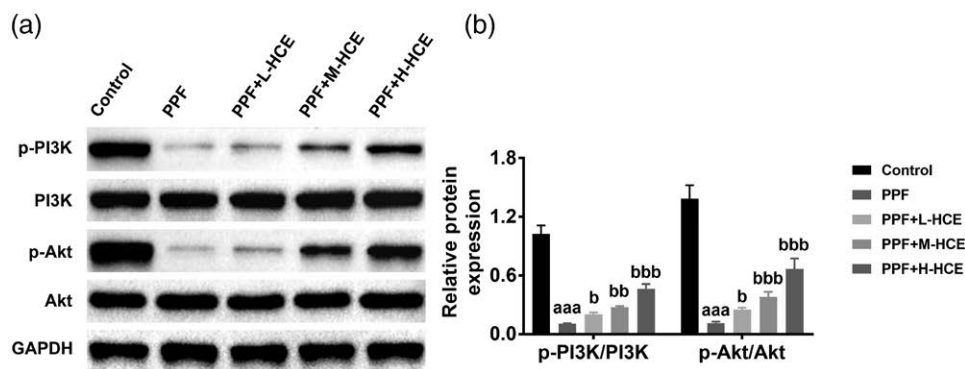


*Houttuynia cordata* extract suppresses neuron cell apoptosis induced by propofol. (a) Flow cytometry assay detected cell apoptosis in control, propofol (100  $\mu$ mol/L), propofol + *H. cordata* extract (25  $\mu$ g/mL), propofol + *H. cordata* extract (50  $\mu$ g/mL), propofol + *H. cordata* extract (100  $\mu$ g/mL) groups. (b) Protein level of Bax, Bcl-2 and cleaved caspase-3 in indicated groups were detected. Three independent experiments were performed. <sup>aaa</sup> $P < 0.001$  versus control; <sup>b</sup> $P < 0.05$ , <sup>bbb</sup> $P < 0.001$  versus propofol. HCE, *Houttuynia cordata* extract; PPF, propofol.

kit. Proteins with different sizes were separated using SDS-PAGE and transferred onto polyvinylidene fluoride membrane (Millipore, Burlington, Massachusetts, USA). Subsequently, the membranes were treated with 5% BSA in 0.1% Tween in TBS buffer for 2 h and then dipped in the following antibody: Bax antibody (1:800 dilution, MAB4601, Millipore), Bcl-2 antibody (1:200 dilution,

ab182858; Abcam, Cambridge, UK), cleaved caspase-3 (1:1000 dilution, ab32042; Abcam), glyceraldehyde 3-phosphate dehydrogenase (1:10000 dilution, ab9485; Abcam), p-PI3K (1:2000 dilution, ab141307; Abcam), PI3K (1:1000 dilution, ab32089; Abcam), p-Akt (1:1000 dilution, ab38449; Abcam) and Akt (1:1000 dilution, ab8805; Abcam). Then, the membranes were cultured

Fig. 4



*Houttuynia cordata* extract promotes activation of PI3K/Akt signaling pathway. (a, b) Protein level of p-PI3K, PI3K, p-Akt, Akt in indicated groups were detected. Three independent experiments were performed. <sup>aaa</sup> $P < 0.001$  versus control; <sup>b</sup> $P < 0.05$ , <sup>bb</sup> $P < 0.01$ , <sup>bbb</sup> $P < 0.001$  versus propofol. HCE, *Houttuynia cordata* extract; PI3K, phosphoinositide 3-kinase; PPF, propofol.

with corresponding secondary antibodies. The bands were visualized with an enhanced chemiluminescent kit. Quantification of the targeted protein was analyzed by ImageJ software.

#### Statistical analysis

The data were analyzed by Graphpad. All data were expressed as means  $\pm$  SD. One-way analysis of variance test was utilized to determine the difference among different groups. Significant differences were recognized at  $P < 0.05$ .

## Results

### *Houttuynia cordata* extract elevates cell viability of primary hippocampal neurons

Rat primary hippocampal neurons were derived from hippocampus of newborn rats. The primary neurons attached after culturing of 24h and started to extend neurites. Then extended neurites as well as neurites with branches and spines (dendrites). After several days of culture, the cell body of neurons aggregated to form a complex and condensed neuron network. Immunofluorescence assay revealed the clear expression of NeuN, verified the nature of cells (Fig. 1a). Then, cells were treated with propofol and *H. cordata* extracts at indicated concentration and measured cell viability in these groups. As known, propofol treatment remarkably reduced the cell viability of neurons. Then, after *H. cordata* extract addition, neuron viability recovered to some extent suggesting the function of *H. cordata* extract in the elevation of primary hippocampal neuron viability (Fig. 1b).

### *Houttuynia cordata* extract reduces neuron cell inflammation induced by propofol

As propofol treatment led to obvious cell inflammation in neurons. We wondered whether the treatment of *H. cordata* extract brought about relief in inflammation. We detected the concentration of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  in neurons treated with propofol of propofol and *H. cordata*

extract. Obviously, propofol induction increased the accumulation of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  in neurons. Then, *H. cordata* extract treatment at different concentrations dramatically reduced the level of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  (Fig. 2a–c). In all, *H. cordata* extracts reduced neuron cell inflammation induced by propofol treatment.

### *Houttuynia cordata* extract suppresses neuron cell apoptosis induced by propofol

Next, we evaluated the effect of *H. cordata* extracts in cell apoptosis in neuron cells. Flow cytometry assay depicted that propofol addition led to obvious cell apoptosis. *H. cordata* extract treatment could significant ameliorate the apoptotic cell number in neuron cells (Fig. 3a). Moreover, the protein level change of Bcl-2 elevation, Bax and cleaved caspase-2 decrease further validated the suppression of cell apoptosis caused by propofol (Fig. 3b).

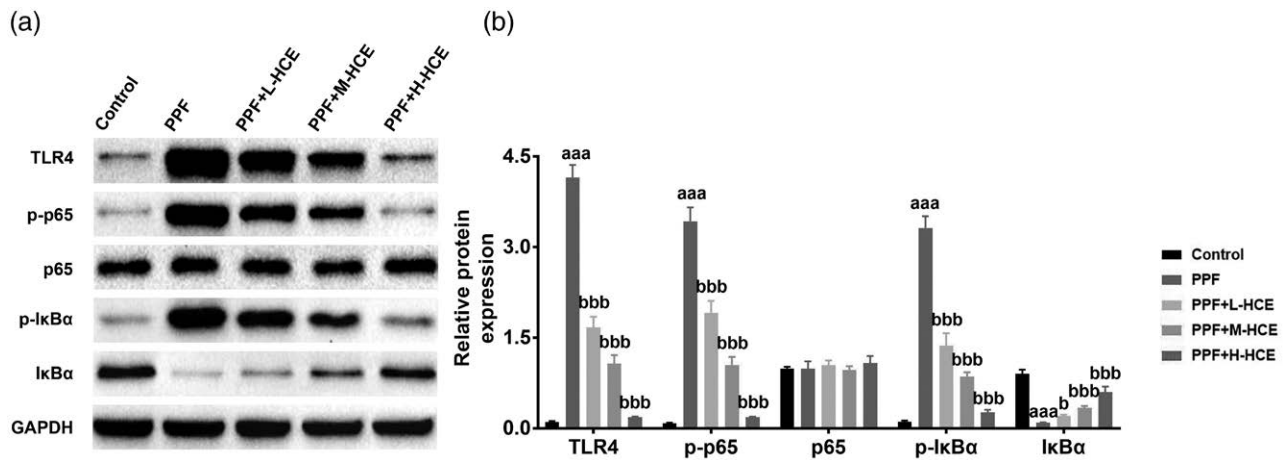
### *Houttuynia cordata* extract promotes activation of PI3K/Akt signaling pathway

The PI3K/AKT pathway was believed to be closely related to neuronal survival since many neurotrophic factors provide beneficial effects in the brain through activating this pathway, to inhibit apoptosis. We noticed the pro-apoptosis effect of *H. cordata* extract before. We attempted to test the effect of *H. cordata* extract on PI3K/AKT pathway. Consistent with our assumption, propofol-treated cells exhibited inhibited p-PI3K and p-Akt levels in neurons; however, *H. cordata* extract supplementation reversed the effect of propofol on PI3K/AKT pathway (Fig. 4a and b). Our results demonstrated the activation effect of *H. cordata* extract on PI3K/AKT pathway.

### *Houttuynia cordata* extract inhibits Toll-like receptor-4/NF- $\kappa$ B signaling pathway

Toll-like receptor-4 (TLR4)/NF- $\kappa$ B signaling mediates many neuroinflammatory diseases. We investigated the potential involvement of TLR4/NF- $\kappa$ B pathway in *H. cordata* extract-mediated propofol-induced neuron

Fig. 5



*Houttuynia cordata* extract inhibits TLR4/NF- $\kappa$ B signaling pathway. (a, b) Protein level of TLR4, I $\kappa$ B $\alpha$ , p-I $\kappa$ B $\alpha$ , p65 and p-p65 in indicated groups were detected. Three independent experiments were performed. <sup>aaa</sup> $P < 0.001$  versus control; <sup>b</sup> $P < 0.05$ , <sup>bbb</sup> $P < 0.001$  versus propofol. HCE, *Houttuynia cordata* extract; NF- $\kappa$ B, nuclear factor kappaB; PPF, propofol; TLR4, Toll-like receptor-4.

injury. In this study, we evaluate the activation state of TLR4, p-I $\kappa$ B and p-p65 in propofol stimulated neurons. As shown in Fig. 5a, propofol treatment effectively enhanced p-I $\kappa$ B, p-p65 and TLR4 expression. However, *H. cordata* extract treatment significantly inhibited p-I $\kappa$ B, p-p65 and TLR4 expression (Fig. 5a and b). Thus, these findings suggest that *H. cordata* extract inhibits TLR4 and NF- $\kappa$ B signaling pathways.

## Discussion

General anesthesia has certain effects on the brain of children whose nervous system is not fully developed, such as short-term memory loss and slow reaction [13]. Though narcotic drugs used at present are generally well tolerated, with short action time and fast metabolism, general anesthetic drugs commonly used clinically can also produce neurotoxic effects on the brain of children during peak periods of development, leading to subsequent persistent and progressive cognitive dysfunction [14]. Interestingly, we found *H. cordata*, a type of perennial herb, had a protective effect on propofol-induced injury of rat hippocampal neurons. Our data, therefore, provide references for anesthetic use in infants and young children.

In this study, we successfully constructed a propofol-induced neuronal cell inflammation model. Propofol was known to lead to the growth cone collapse, loss of synaptic connectivity, axonal transport impairment, and the behavioral deficits of mice [15]. The model has been used in several studies. Previous study showed RTA-408 protected against propofol-induced cognitive impairment in neonatal mice [4]. Additionally, repeated propofol exposure could induce neuronal damage and cognitive impairment in aged rats [16]. Similarly, propofol was successfully used to induce inflammation and apoptosis of hippocampal neurons in rats [17].

The multiple biological activities of *H. cordata* have been widely revealed [18]. *H. cordata* could ameliorate bladder damage and improve bladder symptoms via anti-inflammatory effects in interstitial cystitis rats [8]. *H. cordata* could also attenuate H1N1-induced acute lung injury in mice via inhibition of influenza virus and TLR pathway [19]. *H. cordata* polysaccharide also alleviated intestinal injury and modulated intestinal microbiota in H1N1 virus-infected mice [19,20]. *H. cordata* also has a wide range of other biological activities, such as anticancer, antioxidant and immunomodulatory [19]. In this study, we found *H. cordata* extract had important effects on hippocampal neurons. *H. cordata* extract could increase neuronal activity and reduce neuronal apoptosis in hippocampal neurons. Our data suggest that *H. cordata* could serve as a promising drug for neuropathic disease and provide references for anesthetic use.

PI3K is an important cell signal transduction molecule, which can be activated by stimulation of extracellular signals such as growth factors, cytokines and hormones, to regulate cell proliferation, differentiation, survival and other processes [21]. AKT, also known as protein kinase B, is an important targeted kinase downstream of PI3K. This pathway is closely related to neuronal survival because many neurotrophic factors play a protective role in the brain by activating the pathway, thus inhibiting apoptosis [22]. Similar to our findings, a previous study showed that dexmedetomidine improved propofol-induced neuronal injury in rat hippocampus through the PI3K/Akt pathway [23]. Also, this pathway had protective effects of propofol on intestinal and lung injury induced by intestinal ischemia [24]. The PI3K/Akt pathway also affected propofol-induced postconditioning against focal cerebral ischemia-reperfusion injury in rats [25]. Based on these studies, our data provided evidence that PI3K/

Akt pathway affected the propofol-induced injury of rat hippocampal neurons.

Collectively, we noticed *H. cordata* extract increased neuronal activity, and reduced propofol-induced neuronal inflammation level and neuronal apoptosis. Our data further confirmed *H. cordata* extracts activated PI3K/AKT pathway and suppressed TLR4/NF- $\kappa$ B pathway, therefore protected rat hippocampal neuron injury induced by propofol. Our findings, therefore, provide references for anesthetic use in infants and young children.

## Acknowledgements

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

H.F. designed the study and supervised the data collection. Z.Y. analyzed the data and interpreted the data. L.Y. prepare the article for publication and reviewed the draft of the article. All authors have read and approved the article.

## Conflicts of interest

There are no conflicts of interest.

## Reference

- Song YS, Yang ZZ, Hao YB, Liu WW, Zhang SS, Wang P, et al. Anesthetic effect and safety of sevoflurane combined with propofol in removing tracheobronchial foreign bodies in children. *Eur Rev Med Pharmacol Sci* 2020; **24**:11315–11322.
- Zhao P, Cui Y, Sun L, Sun X. Inhalation of low-dose desflurane prevents the hemodynamic instability caused by target-controlled infusion of remifentanyl and propofol during laparoscopic gynecological surgery: a randomized controlled trial. *Exp Ther Med* 2021; **21**:54.
- Kaur B, Rattalino M. Target-controlled infusion of Propofol and Remifentanyl in a child with recurrent rhabdomyolysis secondary to LPIN1 deficiency. *Paediatr Anaesth* 2020; **30**:726–727.
- Zhang L, Zhou Q, Zhou CL. RTA-408 protects against propofol-induced cognitive impairment in neonatal mice via the activation of Nrf2 and the inhibition of NF- $\kappa$ B p65 nuclear translocation. *Brain Behav* 2021; **11**:e01918.
- Tang YJ, Yang JS, Lin CF, Shyu WC, Tsuzuki M, Lu CC, et al. *Houttuynia cordata* Thunb extract induces apoptosis through mitochondrial-dependent pathway in HT-29 human colon adenocarcinoma cells. *Oncol Rep* 2009; **22**:1051–1056.
- Subhawa S, Chewonarin T, Banjerpongchai R, The effects of *Houttuynia cordata* Thunb and piper ribesiodes wall extracts on breast carcinoma cell proliferation, migration, invasion and apoptosis. *Molecules* 2020; **25**:1196.
- Li W, Fan T, Zhang Y, Fan T, Zhou P, Niu X, He L; *Houttuynia cordata* Thunb. Volatile oil exhibited anti-inflammatory effects in vivo and inhibited nitric oxide and tumor necrosis factor- $\alpha$  production in LPS-stimulated mouse peritoneal macrophages in vitro. *Phytother Res* 2013; **27**:1629–1639.
- Li W, Yang F, Zhan H, Liu B, Cai J, Luo Y, Zhou X. *Houttuynia cordata* extract ameliorates bladder damage and improves bladder symptoms via anti-inflammatory effect in rats with interstitial cystitis, evidence-based complementary and alternative medicine. *eCAM* 2020; **2020**:9026901.
- Wan CF, Zheng LL, Liu Y, Yu X. *Houttuynia cordata* Thunb reverses oxalip-latin-induced neuropathic pain in rat by regulating Th17/Treg balance. *Am J Transl Res* 2016; **8**:1609–1614.
- Xu H, Wang Y, Jurutka PW, Wu S, Chen Y, Cao C, et al. 16 $\alpha$ -Hydroxytram-etenolic acid from *Poria cocos* improves intestinal barrier function through the glucocorticoid receptor-mediated PI3K/Akt/NF- $\kappa$ B pathway. *J Agric Food Chem* 2019; **67**:10871–10879.
- Segarra J, Balenci L, Drenth T, Maina F, Lamballe F. Combined signaling through ERK, PI3K/AKT, and RAC1/p38 is required for met-triggered cortical neuron migration. *J Biol Chem* 2006; **281**:4771–4778.
- Wang M, Suo L, Yang S, Zhang W. CircRNA 001372 reduces inflammation in propofol-induced neuroinflammation and neural apoptosis through PI3KCA/Akt/NF-kappaB by miRNA-148b-3p. *J Invest Surg* 2020:1–11. [Epub ahead of print]
- Yang L, Kang K, Lin Y, Wu Y. Up-regulation of miR-137 can inhibit PTN in target manner to regulate PTN/PTPRZ pathway to prevent cognitive dysfunction caused by propofol. *Am J Transl Res* 2020; **12**:7490–7500.
- Terblanche E, Remington C. Observational study evaluating the nutritional impact of changing from 1% to 2% propofol in a cardiothoracic adult critical care unit. *J Hum Nutr Diet* 2020. [Epub ahead of print]
- Jin W, Zucker M, Pralle A. Membrane nanodomains homeostasis during propofol anesthesia as function of dosage and temperature. *Biochim Biophys Acta Biomembr* 2021; **1863**:183511.
- Mahr N, Bouhake Y, Chopard G, Liu N, Boichut N, Chazot T, et al. Besch, postoperative neurocognitive disorders after closed-loop versus manual target controlled-infusion of propofol and remifentanyl in patients undergoing elective major noncardiac surgery: the randomized controlled postoperative cognitive dysfunction-electroencephalographic-guided anesthetic administration trial. *Anesth Analg* 2020. [Epub ahead of print]
- Prommaban A, Kodchakorn K, Kongtaweert P, Banjerpongchai R. *Houttuynia cordata* Thunb fraction induces human leukemic molt-4 cell apoptosis through the endoplasmic reticulum stress pathway. *Asian Pacific J Cancer Prevention: APJCP* 2012; **13**:1977–1981.
- Toda S. Antioxidative effects of polyphenols in leaves of *Houttuynia cordata* on protein fragmentation by copper-hydrogen peroxide *in vitro*. *J Med Food* 2005; **8**:266–268.
- Ling LJ, Lu Y, Zhang YY, Zhu HY, Tu P, Li H, Chen DF. Flavonoids from *Houttuynia cordata* attenuate H1N1-induced acute lung injury in mice via inhibition of influenza virus and Toll-like receptor signalling. *Phytomedicine* 2020; **67**:153150.
- Zhu H, Lu X, Ling L, Li H, Ou Y, Shi X, et al. *Houttuynia cordata* polysaccharides ameliorate pneumonia severity and intestinal injury in mice with influenza virus infection. *J Ethnopharmacol* 2018; **218**:90–99.
- Zhang Y, Huang N, Chen M, Jin H, Nie J, Shi J, Jin F. Procyanidin protects against 6-hydroxydopamine-induced dopaminergic neuron damage via the regulation of the PI3K/Akt signalling pathway. *Biomed Pharmacother* 2019; **114**:108789.
- Zhang H, Tao J, Zhang S, Lv X. LncRNA MEG3 reduces hippocampal neuron apoptosis via the PI3K/AKT/mTOR pathway in a rat model of temporal lobe epilepsy. *Neuropsychiatr Dis Treat* 2020; **16**:2519–2528.
- Zhu L, Zhang Y, Zhang Z, Ding X, Gong C, Qian Y. Activation of PI3K/Akt/HIF-1 $\alpha$  signaling is involved in lung protection of dexmedetomidine in patients undergoing video-assisted thoracoscopic surgery: a pilot study. *Drug Des Devel Ther* 2020; **14**:5155–5166.
- Zhang W, Zhang JQ, Meng FM, Xue FS. Dexmedetomidine protects against lung ischemia-reperfusion injury by the PI3K/Akt/HIF-1 $\alpha$  signaling pathway. *J Anesth* 2016; **30**:826–833.
- Xiao Y, Zhou L, Tu Y, Li Y, Liang Y, Zhang X, et al. Dexmedetomidine attenuates the propofol-induced long-term neurotoxicity in the developing brain of rats by enhancing the PI3K/Akt signaling pathway. *Neuropsychiatr Dis Treat* 2018; **14**:2191–2206.