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HYPOXIA • ORIGINAL ARTICLE

# Astragaloside IV ameliorates intermittent hypoxia-induced inflammatory dysfunction by suppressing MAPK/NF-κB signalling pathways in Beas-2B cells

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## Abstract

**Purpose** Intermittent hypoxia is a characteristic pathological change in obstructive sleep apnoea (OSA) that can initiate oxidative stress reaction and pro-inflammatory cytokine release. The purpose of this study was to assess the effect and protective mechanism of Astragaloside IV (AS-IV) in intermittent hypoxia-induced human lung epithelial Beas-2B cells.

**Methods** Human lung epithelial Beas-2B cells were exposed to intermittent hypoxia or normoxia in the absence or presence of AS-IV. MTT assay was performed to determine the cell viability. The levels of reactive oxygen species (ROS), lactate dehydrogenase (LDH), malonaldehyde (MDA), and superoxide dismutase (SOD) were measured to evaluate oxidative stress. The levels of cytokines interleukin (IL)-8, IL-1β, and IL-6 were evaluated by enzyme-linked immunosorbent assay and real-time PCR. The expression of Toll-like receptor 4 (TLR4), mitogen-activated protein kinase (MAPK), and nuclear transcription factor-kappa B (NF-κB) signalling pathways was analysed by western blot.

**Results** The results showed that AS-IV significantly reduced the levels of ROS, LDH, MDA, IL-8, IL-1β, and IL-6, and increased the level of SOD in intermittent hypoxia-induced Beas-2B cells. It also suppressed the phosphorylation of MAPKs, including P38, c-Jun N-terminal kinase and extracellular signal-regulated kinase, and inhibited the activation of the NF-κB signalling pathway by reducing the phosphorylation of IκBα and p65.

**Conclusions** AS-IV attenuates inflammation and oxidative stress by inhibiting TLR4-mediated MAPK/NF-κB signalling pathways in intermittent hypoxia-induced Beas-2B cells.

**Keywords** Obstructive sleep apnoea · Intermittent hypoxia · Astragaloside IV · NF-κB

## Introduction

Currently, obstructive sleep apnoea (OSA) is treated with continuous positive airway pressure (CPAP), oral appliance therapy (OAT), and surgical intervention, but none of these options is universally successful [1].

Studies have shown that many oxides are produced during hypoxia–reoxygenation, which leads to oxidative stress in the body [2–4]. These oxides can induce Toll-like receptor 4 (TLR4) to regulate the downstream transcription factors, such as nuclear transcription factor-kappa B (NF-κB), to mediate the production of pro-inflammatory cytokines in OSA patients [5–7]. One study reported that OSA patients show a significant overexpression of TLR4/NF-κB on monocytes [8]. The TLR4/NF-κB pathway has been shown to promote pulmonary inflammation in rats with OSA [9].

Astragaloside IV (AS-IV), the main active compound of *Astragalus membranaceus* Bunge—a type of traditional herb that has long been used to treat numerous diseases—has demonstrated potent protective effects against pulmonary disease, cardiovascular ischemia/reperfusion, diabetic nephropathy, and other diseases [10–16]. In a rat model of hypoxic

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pulmonary hypertension (HPH) induced by chronic hypoxia, AS-IV improved pulmonary function by decreasing the secretion of TNF-α and IL-6 [17]. In obstructive sleep apnea-hypopnea syndrome (OSAHS) patients, after 20 mL Astragalus injection for 20 days, the time of hypoxia during the night was significantly shortened, and the level of vascular endothelial growth factor (VEGF) and apnea-hypopnea index (AHI) were also decreased after treatment [18].

It remains unclear whether or not Astragaloside IV can play a role in IH-induced lung injury. We therefore proposed to use an in vitro model of IH to explore the suppressive effects of AS-IV on the pro-inflammatory mediator overexpression in IH-exposed human lung epithelial Beas-2B cells. We further sought to measure the levels of the TLR4, MAPK, and NF-κB signalling pathway components to clarify the mechanisms by which AS-IV inhibits overoxidation and inflammation.

## Materials and method

### Reagents

AS-IV was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM) and foetal bovine serum (FBS) were purchased from GibcoBRL Co., Ltd. (Grand Island, NY, USA). MTT was purchased from Sigma-Aldrich (St. Louis, Mo, USA). Antibodies against TLR4, p38, JNK, p44/42, phospho-p38, phospho-JNK, phospho-p44/42, myosin light chain (phosphor S20), myosin light chain, and myosin light chain kinase in addition to the NF-κB p50/p65 transcription factor assay kit were purchased from Abcam (Cambridge, MA, USA). Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-6, and IL-1β were purchased from eBioscience (San Diego, CA, USA). Assay kits for ROS, LDH, MDA, and SOD were purchased from Beyotime Biotechnology (Shanghai, China).

### Cell culture

Human lung epithelial Beas-2B cells were purchased from the Cell Culture Unit of Shanghai Science Academy (Shanghai, China) and grown in DMEM supplemented with 10% FBS under 20% $O_2$ and 10% $CO_2$ at 37 °C.

### Exposure of cells to IH

For the IH test group, epithelial Beas-2B cell cultures were exposed to alternating cycles of 1.5% $O_2$ for 30 s followed by 20% $O_2$ for 5 min for a total of 60 cycles (330 s per cycle) at 37 °C in a humidified Lucite chamber as described previously [19]. Cells exposed to repetitive normoxia (alternating cycles of 20% $O_2$ for 30 s followed by 20% $O_2$ for 5 min) served as the control group. To evaluate the effects of AS-IV, the cells were incubated with a series of AS-IV concentrations (0–200 μmol/L) for 24 h before exposure to repetitive normoxia or IH (control and test groups, respectively) without changing the medium.

### Cell viability assay

Cell viability was determined by an MTT assay. Briefly, Beas-2B cells were plated at a density of $1 \times 10^5$ per well in 96-well plates containing DMEM + 10% FBS and then treated with different concentrations (0–200 μmol/L) of AS-IV or DMEM (control medium) for 24 h. Subsequently, the cells were exposed to IH or repetitive normoxia, respectively, and then treated with 10-μl MTT solution for 4 h. The cells were then lysed with 0.04 N HCl in isopropyl alcohol, and the absorbance was read at 570 nm. Cell viability was calculated as follows: cell viability (%) = absorbance of the test group/absorbance of the control group × 100.

### Measurement of ROS, LDH, MDA, and SOD levels in cell culture supernatant

Beas-2B cells were plated in six-well plates at a density of $1 \times 10^6$ per well and treated with AS-IV for 24 h, followed by exposure to IH or repetitive normoxia (test and control groups, respectively). Subsequently, the supernatants were collected and tested using the ROS, LDH, MDA, and SOD assay kits according to the manufacturer's instructions to detect the inhibitory effects of AS-IV on oxide production.

### ELISA

Culture media from the same six-well plates were collected and subjected to ELISA (eBioscience, San Diego, CA) according to the manufacturer's instructions to measure the levels of the pro-inflammatory cytokines IL-8, IL-6, and IL-1β.

### Real-time PCR analysis

The cells from the six-well plates were harvested after IH or normoxia treatment to analyse the levels of IL-8, IL-6, and IL-1β and GAPDH mRNAs. Total RNA was isolated using TRIzol reagent. RNA purity and concentration were determined using a NanoDrop 2000 device (Thermo Scientific, Wilmington, DE, USA). The mRNAs were then reverse transcribed into cDNA using a reverse transcription (RT) qPCR kit according to the manufacturer's instructions. PCR

amplification conditions were as follows: initial denaturation at 95 °C for 15 s, followed by 35 cycles of denaturation at 95 °C for 5 s and annealing at 61 °C for 15 s.

### Measurement of NF-κB p65 activity

After preparing cellular extracts, the NF-κB p65 activity was measured using an NF-κB p50/p65 transcription factor assay kit according to the manufacturer's instructions.

### Western blot analysis

Beas-2B cells were plated in six-well plates at density of $1 \times 10^6$ per well and treated with AS-IV for 24 h, followed by exposure to IH or normoxia (test and control groups, respectively). Then, cells were collected and lysed in lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 1-mmol/L phenylmethylsulphonyl fluoride and protease inhibitors). Cell debris was removed by centrifugation at 15000 rpm and 4 °C for 15 min. After measuring the protein concentration, equal amounts of protein were separated by 12.5% SDS-PAGE and then transferred onto nitrocellulose membranes for western blotting. The membranes were blocked and incubated with primary antibodies at 4 °C overnight, followed by incubation with secondary antibodies at 4 °C for 1 h. Finally, protein expression was detected using the Bio-rad Imaging System (Bio-rad Biosciences, USA).

### Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using Student's *t* test with $p < 0.05$ representing significance. At least three independent experiments were performed.

## Results

### The effect of AS-IV on the viability of IH-exposed Beas-2B cells

The effect of AS-IV on the viability of IH-exposed Beas-2B cells treated with various concentrations (0–200 μmol/L) of AS-IV was tested using an MTT assay. As shown in Fig. 1, AS-IV promoted cell proliferation in a dose-dependent manner. In particular, significant growth promotion was observed for cells treated with 50, 100, and 150 μmol/L of AS-IV. Therefore, these concentrations were selected for further experiments to explore the mechanisms of AS-IV.

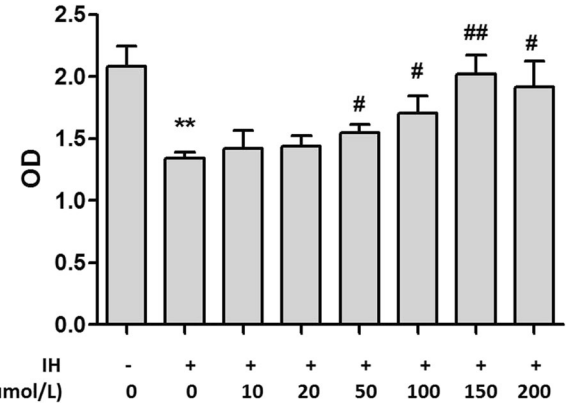


**Fig. 1** AS-IV promoted the proliferation of IH-exposed Beas-2B cells. Beas-2B cells were treated with various concentrations (0–200 μmol/L) of AS-IV or control DMEM for 24 h and then exposed to IH or repetitive normoxia. The effect of AS-IV on cell viability was measured using the MTT assay. Data are presented as the mean ± SEM of at least three independent experiments (**$p < 0.001$ vs. control Beas-2B cell group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. IH-exposed Beas-2B cell group)

### AS-IV suppresses ROS, LDH, and MDA overproduction and promotes SOD secretion in IH-exposed Beas-2B cells

The antioxidative effects of AS-IV were evaluated by measuring the levels of ROS, LDH, MDA, and SOD in IH-exposed Beas-2B cells using corresponding assay kits. As shown in Fig. 2, AS-IV (100 μmol/L) markedly decreased the production of ROS, LDH, and MDA, and significantly increased the secretion of SOD, indicating that AS-IV suppressed oxidative overproduction in IH-exposed Beas-2B cells.

### AS-IV inhibits the secretion of pro-inflammatory cytokines in IH-exposed Beas-2B cells

The potential anti-inflammatory role of AS-IV was evaluated by measuring the overproduction of IL-8, IL-6, and IL-1β in IH-exposed Beas-2B cells by ELISA and real-time PCR. As shown in Fig. 3, the IL-8, IL-6, and IL-1β levels significantly increased in the culture medium of IH-exposed Beas-2B cells. The AS-IV treatment markedly decreased the secretion of these cytokines in these cells. These results indicate that AS-IV can regulate the IH-induced overproduction of pro-inflammatory cytokines.

### AS-IV significantly inhibits TLR4 overexpression in IH-exposed Beas-2B cells

IH stress can activate TLR4 to mediate the initiation of inflammatory and immune defence responses [20, 21]. Therefore,

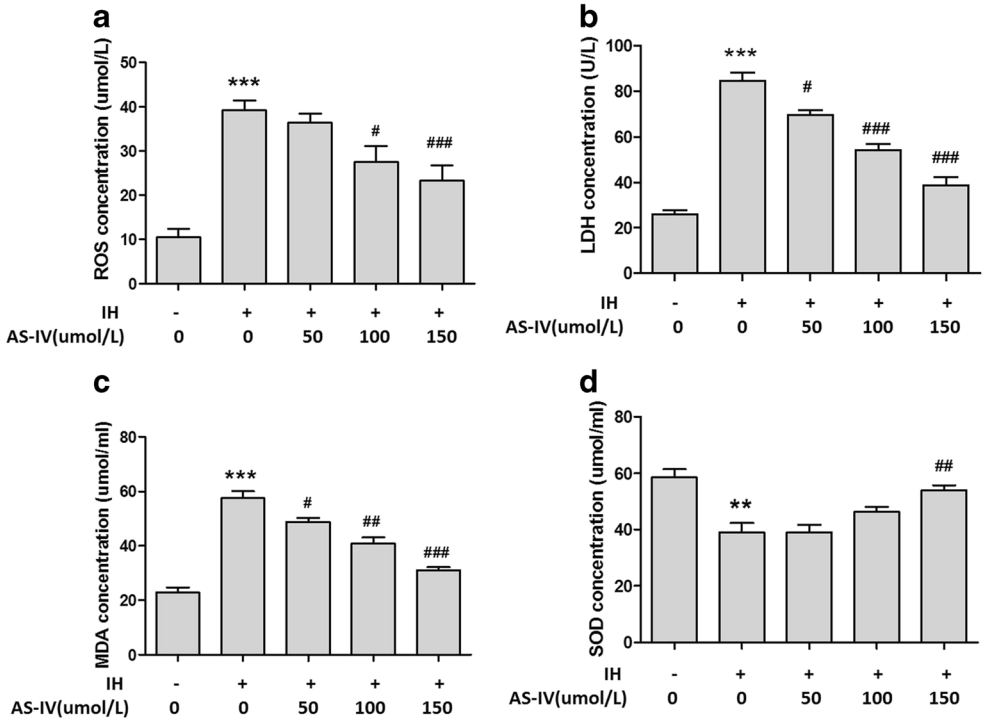


**Fig. 2** AS-IV suppressed ROS, LDH, and MDA overproduction, and promoted SOD secretion in IH-exposed Beas-2B cells. Beas-2B cells were incubated with different concentrations of AS-IV for 24 h and then exposed to IH or repetitive normoxia. The culture supernatants were collected to assay the levels of ROS, LDH, MDA, and SOD. **a**–**c** AS-IV decreased the IH-induced overproduction of ROS, LDH and MDA in the culture supernatants. **b** AS-IV markedly promoted SOD release in the culture supernatants. Data are presented the mean ± SEM of at least three independent experiments (**$p < 0.01$, ***$p < 0.001$ vs. control Beas-2B cell group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. IH-exposed Beas-2B cell group)

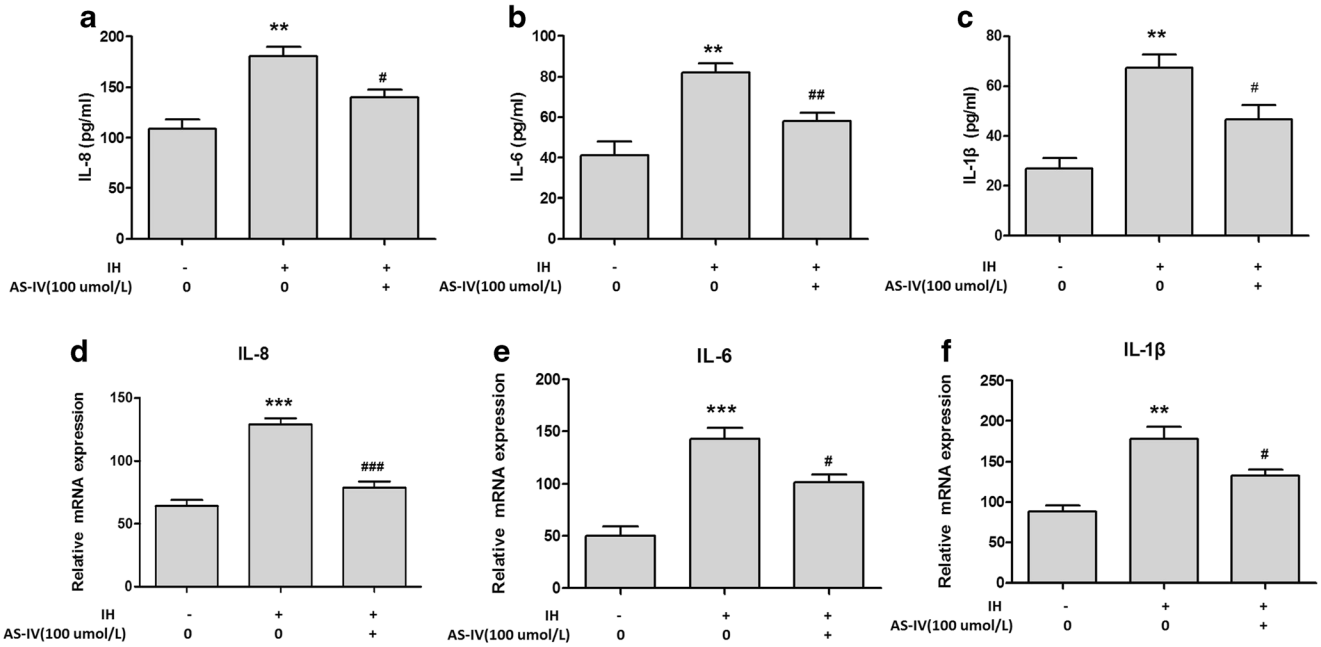


**Fig. 3** AS-IV regulated the secretion of inflammatory cytokines in IH-exposed Beas-2B cells. Beas-2B cells were incubated without or with 100 μmol/L AS-IV for 24 h and then exposed to IH or repetitive normoxia. The cell culture media were collected, and the concentrations of IL-8 (**a**), IL-6 (**b**), and IL-1β (**c**) were determined by ELISA. The cells were harvested to analyse the mRNA expression of IL-8 (**d**), IL-6 (**e**), and IL-1β (**f**) by RT-qPCR (**$p < 0.01$, ***$p < 0.001$ vs. control Beas-2B cell group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.05$ vs. IH-exposed Beas-2B cell group)

we examined the effect of AS-IV on TLR4 protein expression in IH-exposed Beas-2B cells. As shown in Fig. 4, TLR4 expression was significantly upregulated by IH stimulation, but the AS-IV treatment markedly downregulated the expression.

### AS-IV markedly suppresses the activation of the IH-stimulated MAPK signalling pathway in Beas-2B cells

IH-enhanced TLR4 expression induces the production of pro-inflammatory factors via the p38, SAPK/JNK, and ERK1/2-mediated signalling pathways, which are the three parallel pathways of the MAPK signalling pathway [22]. Therefore, we investigated whether the MAPK signalling pathway is involved in the protective effect of AS-IV against IH-induced inflammation in Beas-2B cells.

As shown in Fig. 5, IH markedly increased MAPK activation by increasing the phosphorylation of p38, ERK1/2, and JNK in IH-exposed Beas-2B cells. Although the AS-IV treatment did not significantly affect the production of total p38, ERK1/2, and JNK, 100 μmol/L AS-IV markedly inhibited the overproduction of the phosphorylated forms of p38, ERK1/2, and JNK (phospho-p38, phospho-ERK1/2, and phospho-JNK, respectively). These results indicate that AS-IV can reduce pro-inflammatory cytokine overexpression by inhibiting the activation of the MAPK signalling pathway.

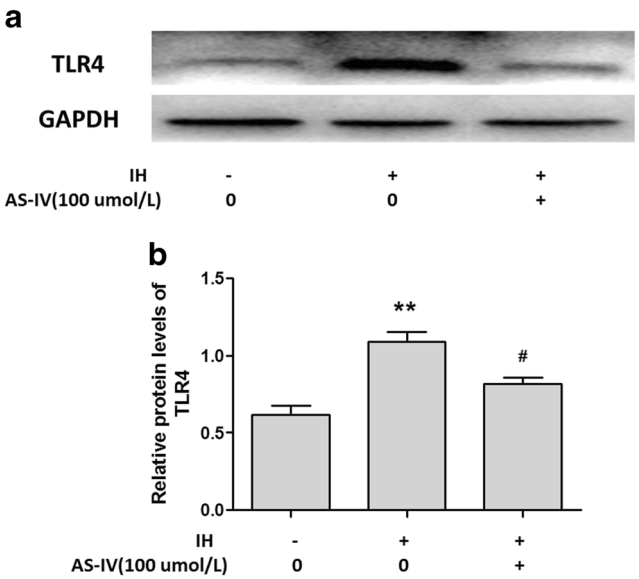


**Fig. 4** AS-IV modified the protein expression of TLR4 in IH-exposed Beas-2B cells. **a** Beas-2B cells were incubated without or with 100 μmol/L AS-IV for 24 h and then exposed to IH or repetitive normoxia. The figure shows a representative western blot of TLR4 in IH-exposed Beas-2B cells treated by AS-IV. **b** Quantification of the amounts of TLR4 relative to GAPDH at 3 h (**$p < 0.01$ vs. control Beas-2B cell group; $^{\#}p < 0.05$ vs. IH-exposed Beas-2B cell group)

### AS-IV significantly inhibits the activation of the IH-induced NF-κB signalling pathway in Beas-2B cells

To further clarify the mechanisms underlying the inhibition of MAPK family members by AS-IV, we studied the effects of AS-IV on the activation of the NF-κB signalling pathway. After the AS-IV treatment (100 μmol/L) and exposure to IH, western blot was performed to examine the levels of phospho-IκBα and phospho-p65 and the production of total IκBα and p65. As demonstrated in Fig. 6a, the NF-κB p65 activity was upregulated in IH-exposed Beas-2B cells. However, the AS-IV treatment significantly inhibited this NF-κB p65 activity. Similarly, as shown in Fig. 6b–e, the production of phospho-IκBα and phospho-p65 significantly enhanced after IH exposure, but the AS-IV treatment (100 μmol/L) markedly inhibited the overproduction of phospho-IκBα and phospho-p65.

## Discussion

IH is one of the most characteristic pathological changes in OSA [5, 23]. When exposed to IH, the lung endothelial cells release various pro-inflammatory factors and oxides, such as IL-6, TNF-a, and ROS, which cause various pathological changes and tissue damage [9]. Our study is the first to demonstrate the suppressive effects of AS-IV on IH-induced oxide overproduction and pro-inflammatory cytokine overexpression in Beas-2B cells. The results showed that AS-IV significantly promoted the proliferation of IH-exposed Beas-2B cells in a dose-dependent manner. It also significantly reduced the levels of ROS, LDH, and MDA; increased the level of SOD; and decreased the mRNA expression and secretion of IL-8, IL-6, and IL-1β in IH-exposed Beas-2B cells.

TLRs play vital roles in innate immunity by defending against invading pathogens when activated [20, 21]. The mRNA and protein levels of TLR4 increased in murine hearts after myocardial ischaemic injury [24]. And under hypoxic stress, HIF-1a binds to the TLR4 promoter region to upregulate the expression of TLR4 [25]. Research showed that TLR4/NF-κB is significantly overexpressed on the monocytes of patients with OSA [8]. In our study, AS-IV demonstrated a significant suppressive effect on IH-activated TLR4 protein overexpression.

The MAPK signalling pathway is activated in response to IH-induced inflammation [22]. The MAPK signalling pathway associated with three parallel pathways, namely the p38, ERK, and JNK signalling pathways [26]. Studies have shown that ERK activation is associated with IH-induced lung epithelial barrier dysfunction [27, 28]. Furthermore, p38

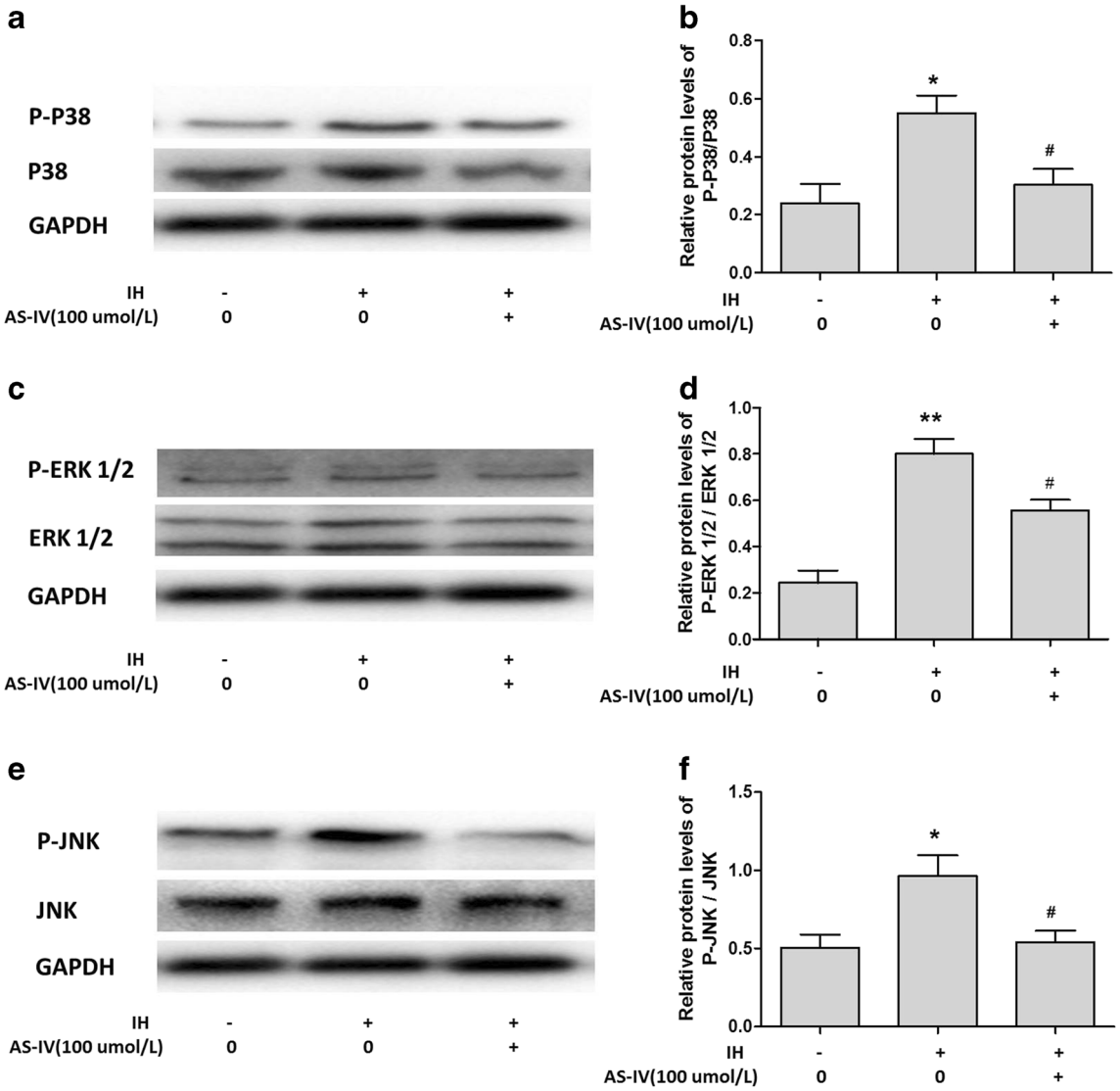


**Fig. 5** AS-IV markedly suppressed the activation of the IH-induced MAPK signalling pathway in IH-exposed Beas-2B cells. **a** Beas-2B cells were incubated without or with 100 μmol/L AS-IV for 24 h and then exposed to IH or repetitive normoxia. The figure shows a representative western blot of phospho-p38 and phospho-p38. **b** Quantification of the amounts of phospho-p38 relative to p38. **c** The figure shows a representative western blot of phospho-ERK1/2 and ERK1/2. **d** Quantification of the amounts of phospho-ERL1/2 relative to ERK1/2. **e** The figure shows a representative western blot of phospho-JNK and JNK. **b** Quantification of the amounts of phospho-JNK relative to JNK (*$p < 0.05$, **$p < 0.01$ vs. control Beas-2B cell group; $^{\#}p < 0.05$ vs. IH-exposed Beas-2B cell group)

activation is associated with the production of inflammatory mediators that initiate the recruitment and activation of leucocytes; p38 also regulates the expression of various inflammation-related genes, such as those encoding for TNF-α and IL-6 [29, 30]. JNK activation by IH, inflammatory cytokines, and other stimulation induces the expression of pro-inflammatory cytokines [31, 32]. In our study, AS-IV treatment inhibited the phosphorylation of p38, ERK, and JNK in IH-exposed Beas-2B cells. This result suggests that the inhibition of MAPK phosphorylation is the mechanism by which AS-IV attenuated inflammation in IH-exposed Beas-2B cells.

NF-κB is an important downstream target of the MAPK signalling pathways that regulates various genes encoding for pro-inflammatory mediators of inflammatory and immune responses [33, 34]. One study demonstrated the involvement of the TLR4/NF-kB signalling pathway activation in lung tissue inflammation in IH-exposed rats [9]. In our study, AS-IV treatment significantly suppressed the IH-induced phosphorylation of IκBα and further inhibited NF-κB p65 activation and reduced p65 phosphorylation.

There are several shortcomings in this study that should be acknowledged. First, the in vitro model used in this study is limited. It will be important to explore the use of AS-IV for the

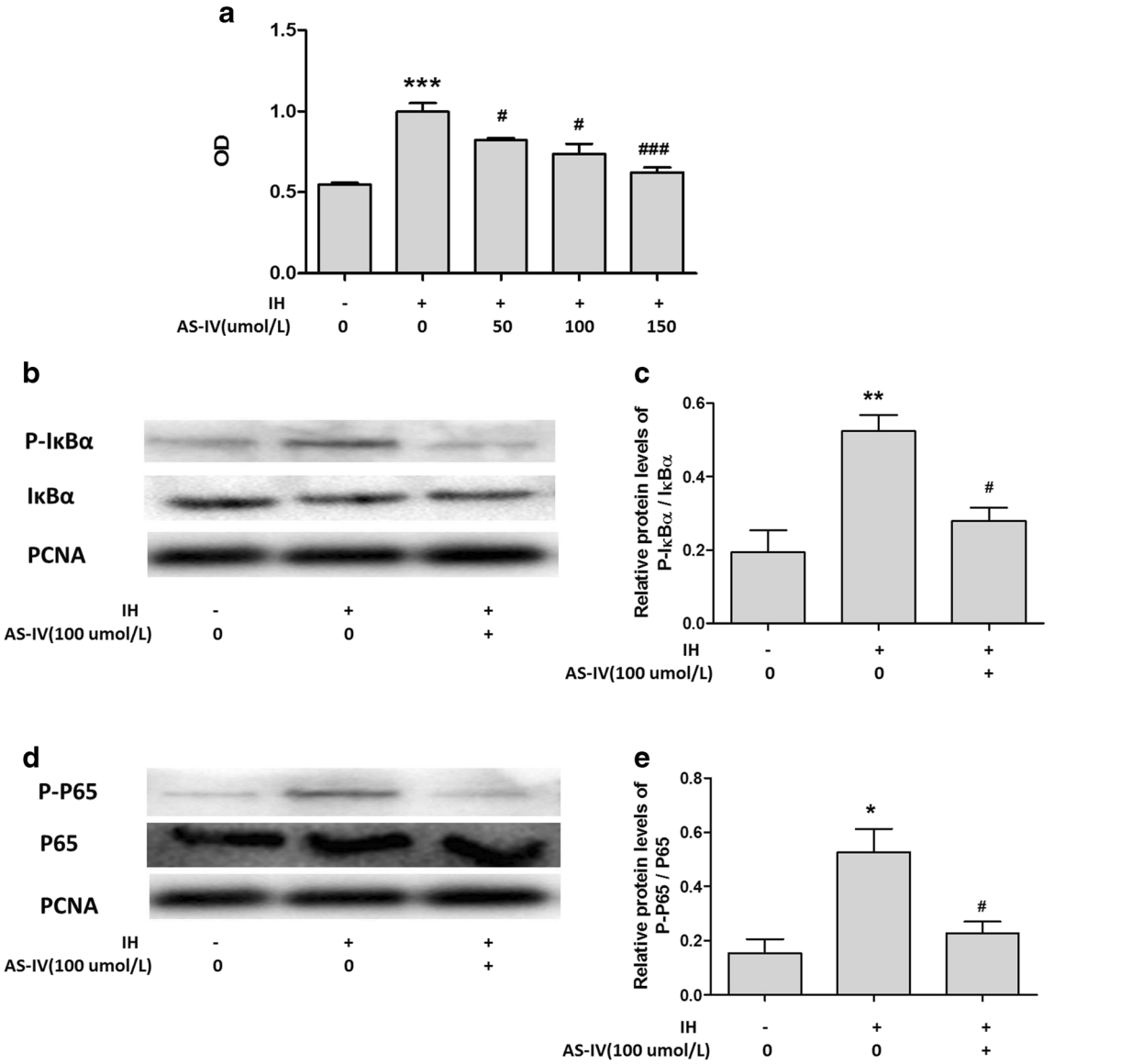


**Fig. 6** Effect of AS-IV on the NF-κB signalling pathway in IH-exposed Beas-2B cells. **a** AS-IV upregulated the activity of the NF-κB transcription factor p65. **b** Beas-2B cells were incubated without or with 100 μmol/L AS-IV for 24 h and then exposed to IH or repetitive normoxia. The figure shows a representative western blot of phospho-IκBα and IκBα. **c** Quantification of the amounts of phospho-IκBα relative to IκBα. **d** The figure shows a representative western blot of phospho-p65 and p65. **e** Quantification of the amounts of phospho-p65 relative to p65. Data are presented as the mean ± SEM of at least three independent experiments ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control Beas-2B cell group; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. IH-exposed Beas-2B cell group)

treatment of IH in a murine model and to consider the differences between in vitro and in vivo findings. Furthermore, the feasibility of using AS-IV for the treatment of patients with OSAHS needs to be explored.

## Conclusion

In our study, AS-IV was shown to attenuate the oxidative response and inflammation in IH-exposed Beas-2B cells. Our results also demonstrated that AS-IV inhibits IH-induced TLR4-mediated MAPK/NF-κB signalling pathways, indicating that this may be the mechanism underlying the protective effect of AS-IV against oxidative and inflammatory responses.

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## Compliance with ethical standards

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

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Abbreviations** *IH,* Intermittent hypoxia; *OSA,* Obstructive sleep apnoea; *AS-IV,* Astragaloside IV; *CPAP,* Continuous positive airway pressure; *ROS,* Reactive oxygen species; *MDA,* Malonaldehyde; *LDH,* Lactate dehydrogenase; *SOD,* Superoxide dismutase; *TLR4,* Toll-like receptor 4; *MAPK,* Mitogen-activated protein kinase; *JNK,* c-Jun N-terminal kinase; *ERK,* Extracellular signal-regulated kinase; *IL-8,* Interleukin-8; *IL-6,* Interleukin-6; *IL-1β,* Interleukin-1β; *HPH,* Hypoxic pulmonary hypertension; *OSAHS,* Obstructive sleep apnea-hypopnea syndrome; *VEGF,* Vascular endothelial growth factor; *AHI,* Apnea-hypopnea index

## References


1. Raggi A, Ferri R (2012) Cognitive evoked potentials in obstructive sleep apnea syndrome: a review of the literature. Rev Neurosci 23: 311–323
2. Jia W, Jian Z, Li J, Luo L, Zhao L, Zhou Y, Tang F, Xiao Y (2016) Upregulated ATF6 contributes to chronic intermittent hypoxia-afforded protection against myocardial ischemia/reperfusion injury. Int J Mol Med 37:1199–1208
3. Passali D, Corallo G, Yaremchuk S, Longini M, Proietti F, Passali GC, Bellussi L (2015) Oxidative stress in patients with obstructive sleep apnea syndrome. Acta Otorhinolaryngol Ital 35:420–425
4. Xu Q, Du J, Ling X, Lu Y (2017) Evaluation of MIh scoring system in diagnosis of obstructive sleep apnea syndrome. Med Sci Monit 23:4715–4722
5. Zhang J, Zheng L, Cao J, Chen B, Jin D (2015) Inflammation induced by increased frequency of intermittent hypoxia is attenuated by tempol administration. Braz J Med Biol Res 48:1115–1121
6. Baud V, Collares D (2016) Post-translational modifications of RelB NF-kB subunit and associated functions. Cells 5:22
7. Israel LP, Benharoch D, Gopas J, Goldbart AD (2013) A pro-inflammatory role for nuclear factor kappa B in childhood obstructive sleep apnea syndrome. Sleep 36:1947–1955
8. Akinnusi M, Jaoude P, Kufel T, El-Solh AA (2013) Toll-like receptor activity in patients with obstructive sleep apnea. Sleep Breath 17:1009–1016
9. Yang JJ, Wang SJ, Gao X, Wang B, Dong YT, Bai Y, Chen Y, Gong JN, Huang YQ, An DD (2018) Toll-like receptor 4 (TLR-4) pathway promotes pulmonary inflammation in chronic intermittent hypoxia-induced obstructive sleep apnea. Med Sci Monit 7:7152–7161
10. He Y, Xi J, Zheng H, Zhang Y, Jin Y, Xu Z (2012) Astragaloside IV inhibits oxidative stress-induced mitochondrial permeability transition pore opening by inactivating GSK-3 via nitric oxide in H9c2 cardiac cells. Oxidative Med Cell Longev 2012:1–9
11. Xu XL, Ji H, Gu SY, Shao Q, Huang QJ, Cheng YP (2007) Modification of alterations in cardiac function and sarcoplasmic reticulum by astragaloside IV in myocardial injury in vivo. Eur J Pharmacol 568:203–212
12. Du Q, Chen Z, Zhou LF, Zhang Q, Huang M, Yin KS (2008) Inhibitory effects of astragaloside IV on ovalbumin-induced chronic experimental asthma. Can J Physiol Pharmacol 86:449–457
13. Gu X, Jiang D, Wang Y, Du Q, Cai J (2012) Effects of astragaloside IV on eosinophil activation induced by house dust mite allergen. Mol Med Rep 6:115–120
14. Huang X, Tang L, Wang F, Song G (2014) Astragaloside IV attenuates allergic inflammation by regulation Th1/Th2 cytokine and enhancement CD4(+)CD25(+)Foxp3 T cells in ovalbumin-induced asthma. Immunobiology 219:565–571
15. Chen HC, Chen CA, Guh JY, Chang JM, Shin SJ, Lai YH (2000) Altering expression of alpha3beta1 integrin on podocytes of human and rats with diabetes. Life Sci 67:2345–2453
16. Nlandu Khodo S, Dizin E, Sossauer G, Szanto I, Martin PY, Feraille E, Krause KH, de Seigneux S (2012) NADPH-oxidase 4 protects against kidney fibrosis during chronic renal injury. J Am Soc Nephrol 23:1967–1976
17. Zhang X, Chen J, Xu P, Tian X (2018) Protective effects of astragaloside IV against hypoxic pulmonary hypertension. Medchemcomm 9:1715–1721
18. Shang N, Gu XY, Jiang DS, Li YL (2008) The effect of Astragalus injection in vascular endothelial growth factor (VEGF) of obstructive sleep apnea-hypopnea syndrome patients. Jiangsu Med J 34: 620–621
19. Yuan G, Nanduri J, Khan S, Semenza GL, Prabhakar NR (2008) Induction of HIF-1alpha expression by intermittent hypoxia: involvement of NADPH oxidase, Ca2 signaling, prolyl hydroxylases, and mTOR. J Cell Physiol 217:674–685
20. Merry HE, Phelan P, Doak MR, Zhao M, Hwang B, Mulligan MS (2015) Role of toll-like receptor-4 in lung ischemia-reperfusion injury. Ann Thorac Surg 99:1193–1199
21. Wang Y, Chai Y, He X, Ai L, Sun X, Huang Y, Li Y (2017) Intermittent hypoxia simulating obstructive sleep apnea causes pulmonary inflammation and activates the Nrf2/HO-1 pathway. Exp Ther Med 14:3463–3470
22. Shang J, Yang YY, Guo XL, Liu HG (2013) Ang II type 1 receptor expression in rat aorta exposed to chronic intermittent hypoxia: Effects of p38MAPK and ERK1/2 signaling. Chin Med J 126: 3264–3269
23. Zhou YQ, Ye JY (2016) Personalized combined modality therapy based on pathophysiology of obstructive sleep apnea hypopnea syndrome. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 51: 877–880 [in Chinese]
24. Satoh M, Nakamura M, Akatsu T, Iwasaka J, Shimoda Y, Segawa I, Hiramori K (2003) Expression of Toll-like receptor 4 is associated with enteroviral replication in human myocarditis. Clin Sci (Lond) 104:577–584
25. Kim SY, Choi YJ, Joung SM, Lee BH, Jung YS, Lee JY (2010) Hypoxic stress up-regulates the expression of Toll-like receptor 4 in macrophages via hypoxia-inducible factor. Immunology 129:516–524
26. Son Y, Cheong YK, Kim NH, Chung HT, Kang DG, Pae HO (2011) Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? J Signal Transduct 2011:792639
27. Smith KA, Yuan JX (2014) Breaking barriers in obstructive sleep apnea. Focus on "Intermittent hypoxia-induced endothelial barrier dysfunction requires ROS-dependent MAP kinase activation.". Am J Phys Cell Physiol 15:C724–C725
28. Makarenko VV, Usatyuk PV, Yuan G, Lee MM, Nanduri J, Natarajan V, Kumar GK, Prabhakar NR (2014) Intermittent hypoxia-induced endothelial barrier dysfunction requires ROS-dependent MAP kinase activation. Am J Phys Cell Physiol 15: C745–C752
29. Ho FM, Lai CC, Huang LJ, Kuo TC, Chao CM, Lin WW (2004) The anti-inflammatory carbazole, LCY-2-CHO, inhibits lipopolysaccharide-induced inflammatory mediator expression through inhibition of the p38 mitogen-activated protein kinase signaling pathway in macrophages. Br J Pharmacol 141:1037–1047
30. Klintman D, Li X, Santen S, Schramm R, Jeppsson B, Thorlacius H (2005) p38 mitogen-activated protein kinase-dependent chemokine production, leukocyte recruitment, and hepatocellular apoptosis in endotoxemic liver injury. Ann Surg 242:830–838

31. Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, Leisten JC, Motiwala A, Pierce S, Satoh Y, Bhagwat SS, Manning AM, Anderson DW (2001) SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. Proc Natl Acad Sci U S A 98:13681–13686
32. Newton K, Dixit VM (2012) Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol 4:1–19
33. Dai JN, Zong Y, Zhong LM, Li YM, Zhang W, Bian LG, Ai QL, Liu YD, Sun J, Lu D (2011) Gastrodin inhibits expression of inducible NO synthase, cyclooxygenase-2 and proinflammatory cytokines in cultured LPS-stimulated microglia via MAPK pathways. PLoS One 6:e21891
34. Baeuerle PA, Baltimore D (1996) NF-kappa B: ten years after. Cell 87:13–20