Propofol selectively inhibits nuclear factor-κB activity by suppressing p38 mitogen-activated protein kinase signaling in human EA.hy926 endothelial cells during intermittent hypoxia/reoxygenation

DONGLIANG LI[1*], CHUNLING WANG[1*], NING LI[2] and LI ZHANG[1]

[1]Department of Anesthesiology, Qilu Hospital, Shandong University, Jinan, Shandong 250012;
[2]School of Public Health, Jining Medical University, Jining, Shandong 272067, P.R. China

Received June 12, 2013; Accepted February 4, 2014

DOI: 10.3892/mmr.2014.1946

Abstract. Intermittent hypoxia/reoxygenation (IHR) induces proinflammatory cytokines, contributing to the pathogenic process of atherosclerosis associated with obstructive sleep apnea (OSA). Two transcription factors, nuclear factor-κB (NF-κB) and hypoxia-inducible factor-1 (HIF-1), have been indicated to mediate proinflammatory cytokines during IHR. The anti-inflammatory effects of propofol have attracted increasing attention in regard to the treament of multiple diseases associated with inflammation. The present study examined whether propofol inhibits NF-κB and HIF-1 activity in vascular endothelial cells during IHR. EA.hy926 endothelial cells were exposed to IHR for 64 cycles with or without propofol treatment. Gene knockdown by transfection of siRNA against p38 mitogen-activated protein kinase (MAPK) was also used to investigate the molecular mechanisms. Compared with the control group, IHR exposure significantly induced the activation of NF-κB and HIF-1, enhanced the mRNA expression of proinflammatory cytokines and increased the activation of p38 MAPK. Propofol dose-dependently inhibited the IHR-induced activation of NF-κB, but did not change the activation of HIF-1, which was accompanied by decreased levels of proinflammatory cytokines. In addition, IHR-induced p38 MAPK activity was attenuated by propofol in a similar manner to the reduction in NF-κB activity. Furthermore, knockdown of p38 MAPK with siRNA significantly reduced the IHR-induced activation of NF-κB, while not affecting HIF-1. These data demonstrate that propofol selectively attenuates the IHR-induced activation of NF-κB, but not HIF-1, in vascular endothelial cells, and these beneficial effects are likely to be based on the inhibition of the p38 MAPK signaling pathway. Propofol may have the potential to prevent atherosclerosis in patients with OSA by inhibiting NF-κB-mediated inflammation in the vascular endothelium.

Correspondence to: Dr Li Zhang, Department of Anesthesiology, Qilu Hospital, Shandong University, 107 Wenhuaxi Road, Jinan, Shandong 250012, P.R. China
E-mail: zhangl-sdu@hotmail.com

*Contributed equally

Key words: intermittent hypoxia, nuclear factor-κB, p38 mitogen-activated protein kinase, propofol

Introduction

Obstructive sleep apnea (OSA) is characterized by intermittent hypoxia/reoxygenation (IHR) as a result of repetitive episodes of complete or partial obstructions of the upper airway during sleep. IHR is an independent risk factor for the development of coronary and cerebral vascular diseases, two common consequences of atherosclerosis (1-3). The mechanisms by which hypoxic signaling accelerates the initiation and progression of atherosclerosis have yet to be fully elucidated.

Nuclear factor-κB (NF-κB) is a transcription factor that has crucial roles in inflammation, immunity, cell proliferation and apoptosis (4-6). Activation of NF-κB is controlled by the inhibitor of κB (I-κB), which retains NF-κB in the cytoplasm (7). Emerging evidence has revealed that the activation of NF-κB in the endothelium may contribute to the pathogenic process of atherosclerosis associated with IHR (8,9). NF-κB-mediated inflammatory pathways have integrated roles in classic atherosclerosis induced by a high-cholesterol diet (9). Patients with OSA have increased NF-κB activity in circulating neutrophils and monocytes, and elevated serum levels of NF-κB-dependent gene products (10-12). Furthermore, using an *in vitro* model in cultured cells exposed to repetitive hypoxia/reoxygenation, previous studies have demonstrated a selective and dose-dependent activation of NF-κB compared with adaptive hypoxia-inducible factor-1 (HIF-1)-dependent pathways, and the p38 mitogen-activated protein kinase (MAPK) signaling pathway is believed to mediate the activation of NF-κB during IHR (7).

Propofol (2,6-diisopropylphenol) is a potent intravenous hypnotic agent widely used for the induction and maintenance of anesthesia. In addition, propofol exhibits anti-inflammatory properties by decreasing the production of proinflammatory cytokines, altering the production of nitric oxide and

inhibiting neutrophil function (13). A study reported that propofol inhibited the activation of p38 MAPK to exert its anti-inflammatory effect (14). However, it remains to be elucidated whether propofol inhibits NF-κB and HIF-1 activity in the vascular endothelial cells during IHR. In the present study, an *in vitro* model of human vein endothelial cells was employed to mimic IHR events and evaluate the effects of propofol on the IHR-induced activation of NF-κB and HIF-1 and their molecular mechanisms.

Materials and methods

Cell culture and IHR. The human endothelial cell line EA.hy926, generated from a fusion of primary human umbilical vein endothelial cells (HUVECs), was purchased from the Cell Library of Shanghai Institutes for Biological Sciences (Shanghai, China). The EA.hy926 cells were grown and maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS) and kept in a humidified incubator at 5% CO_2. For all experiments, the cells were grown to 50-70% confluency and starved for 1 day in 1% FBS medium prior to the different treatments. IHR exposure was performed in a computer-controlled incubator chamber connected to a BioSpherix OxyCycler (Biospherix, Redfield, NY, USA), as previously described (8). The cells were maintained at 37˚C and 5% CO_2 in the hypoxic chamber, in which the O_2 levels were shifted between 1% for 10 min and 21% for 5 min. The cells were exposed to IHR for 64 cycles based on a previous study in which such cycles of IHR were determined to sufficiently induce the activation of NF-κB in HUVECs (8). The cells in the control group were maintained in normoxic conditions at 21% O_2 and 5% CO_2.

Propofol treatment. Pure propofol was purchased from Sigma-Aldrich (St. Louis, MO, USA) to exclude the effect of lipid emulsion. Prepared propofol was added to the medium at different concentrations of 0, 25, 50 or 100 μM for 30 min prior to IHR exposure, and kept in the medium throughout the entire experiment. This dose range for propofol was selected from a previous study and considered as the range of concentrations that was clinically relevant (15). Cells that were not treated with either propofol or IHR were used as a control. At the end of the experiments, a luciferase reporter assay, western blot analysis or quantitative polymerase chain reaction (qPCR) were performed, as described below.

Knockdown of p38 MAPK by siRNA. siRNA specifically targeting p38 MAPK and a control siRNA were purchased from Dharmacon (Lafayette, CO, USA). The cells were grown to 50% confluence in antibiotic-free medium, and p38 siRNA or control siRNA were transfected using Lipofectamine® transfection reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The cells were incubated at 37˚C for 48 h to allow for maximal knockdown of the target gene and then exposed to the indicated cycles of IHR. A luciferase reporter assay or western blot analysis was performed, as described below.

Luciferase reporter assays for NF-κB and HIF-1 activity. Luciferase reporter assays were conducted, as described previously (7). Briefly, the cells were grown to 50-70% confluence on culture dishes and transiently transfected with NF-κB or HIF-1 luciferase reporter constructs and then co-transfected with a constitutively active Renilla luciferase reporter construct (pSV40-Renilla; Promega Corp., Madison, WI, USA). At 24 h post-transfection, the cells were lysed in luciferase cell lysis buffer (Promega Corp., Fitchburg, WI, USA). The luciferase activity was assessed by addition of an excess of luciferin/adenosine triphosphate (Promega Corp., Fitchburg, WI, USA) and luminometry (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany). All the luciferase reporter values were normalized to the Renilla luciferase activity and expressed as a fold induction relative to the control group.

Western blot analysis. Cell homogenates were separated by 10% SDS-PAGE and transferred to a polyvinylidene fluoride membrane. The membrane was blocked in Tris-buffered saline Tween-20 (TBST) with 5% skimmed milk and incubated overnight with primary rabbit polyclonal anti-I-κBα, mouse monoclonal anti-phosphorylated I-κBα (1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), rabbit monoclonal anti-p38 MAPK, rabbit monoclonal anti-phosphorylated p38 MAPK (1:1,000 and 1:500, respectively; Cell Signaling Technology, Inc., Danvers, MA, USA) and mouse monoclonal anti-β-actin (1:200; Santa Cruz Biotechnology, Inc.) antibodies. Next, the membrane was incubated for 1 h with secondary antibodies diluted with TBST. The signals of the detected proteins were visualised by an enhanced chemiluminescence (ECL) reaction system (Millipore, Billerica, MA, USA) and quantified by ImageJ software. (National Institutes of Health, Bethesda, MD, USA)

qPCR. Total RNA was prepared from the cells that remained in the wells by extraction with TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) and purification using an RNeasy Mini kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. RNA with an A260/280 ratio between 1.8 and 2.0 was used for reverse transcription using the qScript cDNA kit (Quanta BioSciences, Gaithersburg, MD, USA). qPCR was performed in the ABI prism 7000 Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA) using SYBR® Green (SuperArray Bioscience, Valencia, CA, US). The primer sequences were as follows: Tumor necrosis factor α (TNF-α) forward, 5'-CGAGTGACAAGCCTGTAGC-3' and reverse, 5'-GGTGTG GGTGAGGAGCACAT-3'; interleukin-1β (IL-1β) forward, 5'-AAACAGATGAAGTGCTCCTTCCAGG-3' and reverse, 5'-TGGAGAACACCACTTGTTGCTCCA-3'; interleukin-6 (IL-6) forward, 5'-AAATGCCAGCCTGCTGACGAAC-3' and reverse, 5'-AACAACAATCTGAGGTGCCCATGCTAC-3'; and GAPDH forward, 5'-TGGGCTACACTGAGCACCAG-3' and reverse, 5'-GGGTGTCGCTGTTGAAGTCA-3'. The values were normalized to GAPDH and the final concentration of mRNA was calculated using the formula $x = 2^{-\Delta\Delta Ct}$, where x is the fold difference relative to the control.

Statistical analysis. Values are expressed as the mean ± standard errpr from at least three independent experiments. Each treatment was performed in the triplicate culture wells.

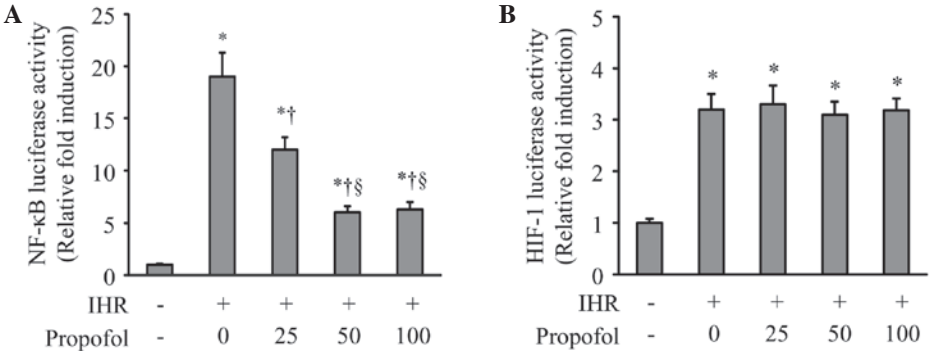

Figure 1. Effects of propofol on the activation of NF-κB and HIF-1 as measured by the luciferase reporter assay. Propofol selectively inhibited (A) IHR-induced activation of NF-κB in a dose-dependent manner but did not affect (B) activation of HIF-1 in HUVECs. IHR(-)/propofol(-) served as the control. The values are presented as the mean ± standard error (n=3-5 for each group). *P<0.05 vs. control; †P<0.05 vs. IHR alone; and §P<0.05 vs. IHR+25 μM propofol. NF-κB, nuclear factor-κB; HIF-1, hypoxia-inducible factor-1; IHR, intermittent hypoxia/reoxygenation; HUVEC, human umbilical vein endothelial cell.

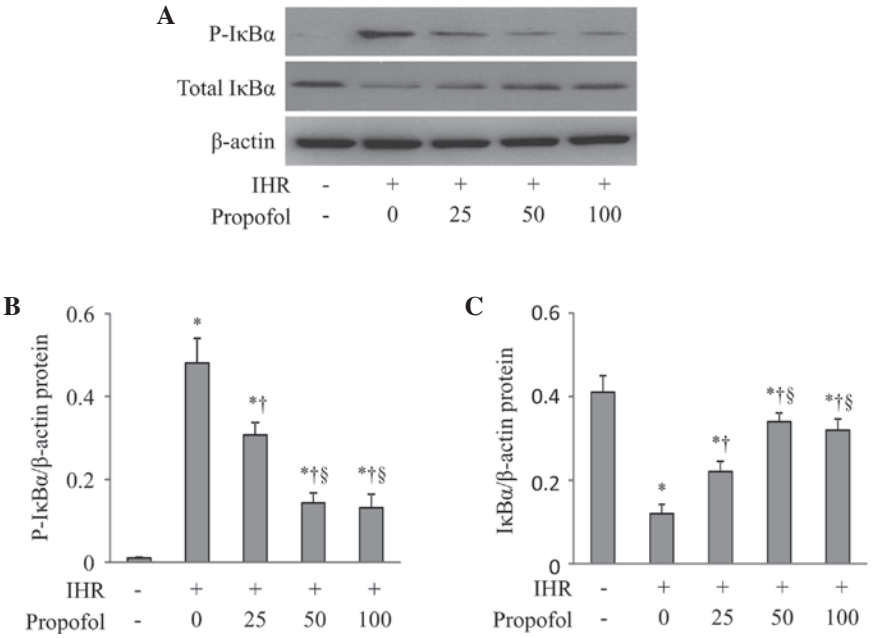

Figure 2. (A) Representative western blot images and quantitative comparison of protein levels for (B) phosphorylated and (C) total IκBα in each group. Propofol prevented the IHR-induced phosphorylation and degradation of IκBα. IHR(-)/propofol(-) served as the control. The values are presented as the mean ± standard error (n=4 for each group). *P<0.05 vs. control; †P<0.05 vs. IHR alone; and §P<0.05 vs. IHR + 25 μM propofol. P-IκBα, phosphorylated IκBα. IκBα, inhibitor of nuclear factor-κB; IHR, intermittent hypoxia/reoxygenation.

Statistically significant values were tested by one-way analysis of variance followed by Bonferroni's post-hoc test for multiple comparisons. P<0.05 was used to indicate a statistically significant difference.

Results

Effects of propofol on IHR-induced NF-κB and HIF-1 activity. Using luciferase reporter assays, the NF-κB and HIF-1 signaling pathway activity in HUVECs was measured in response to propofol treatment. As shown in Fig. 1, 64 cycles of IHR significantly induced the activation of NF-κB and HIF-1 compared with the control group. Propofol at 25 and 50 μM dose-dependently inhibited the NF-κB activity compared with IHR alone. High-dose propofol (100 μM) did not further reduce NF-κB activity (Fig. 1A). By contrast, neither a high nor a low dose of propofol affected the IHR-induced HIF-1 activity (Fig. 1B).

To examine whether propofol inhibited the activation of NF-κB through regulating its inhibitor, I-κB, in the cytoplasm, the protein levels of total and phosphorylated I-κBα were assessed (Fig. 2). IHR alone caused a significant increase in I-κBα phosphorylation (Fig. 2B) and a decrease in the total I-κBα levels (Fig. 2C), and these changes were prevented by propofol in a dose-dependent manner.

Effects of propofol on IHR-induced proinflammatory cytokines. Due to NF-κB being a key component in the regulation of inflammation, the present study examined whether propofol treatment reduces IHR-induced expression of proinflammatory cytokines in HUVECs. qPCR demonstrated that the mRNA expression of the proinflammatory cytokines TNF-α, IL-1β

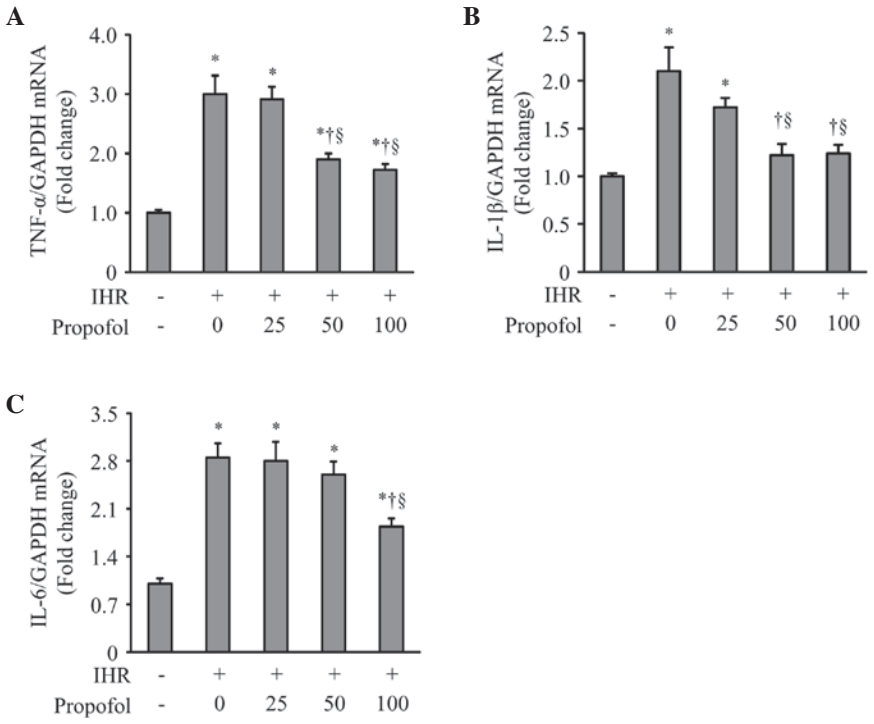

Figure 3. Effects of propofol on proinflammatory cytokines. IHR induced increases in mRNA expression of TNF-α, IL-1β and IL-6 in HUVECs that were reduced by propofol at 50 or 100 μM. IHR(-)/propofol(-) served as the control. The values are presented as the mean ± standard error (n=5 for each group). *P<0.05 vs. control; †P<0.05 vs. IHR alone; and §P<0.05 vs. IHR + 25 or 50 μM propofol. IHR, intermittent hypoxia/reoxygenation; TNF-α, tumor necrosis factor; IL, interleukin; HUVEC, human umbilical vein endothelial cells.

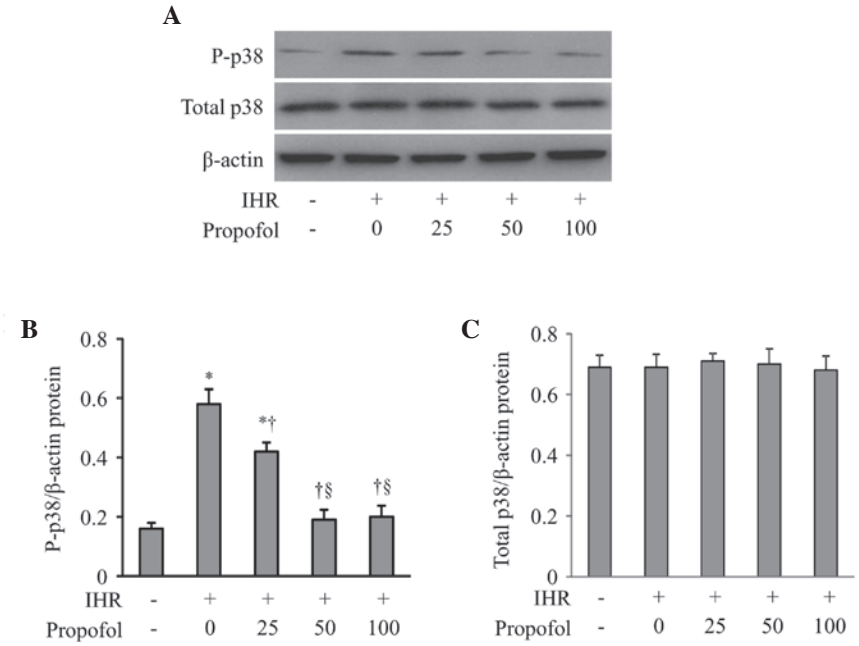

Figure 4. (A) Representative western blot images and quantitative comparison of protein levels for (B) phosphorylated and (C) total p38 MAPK in each group. Propofol dose-dependently reduced the protein level of the phosphorylated p38 MAPK, but had no effect on the total p38 MAPK in HUVECs exposed to IHR. IHR(-)/propofol(-) served as the control. The values are presented as the mean ± standard error (n=4 for each group). *P<0.05 vs. control; †P<0.05 vs. IHR alone; and §P<0.05 vs. IHR + 25 μM propofol. P-p38, phosphorylated p38 MAPK; MAPK, mitogen-activated protein kinase; HUVEC, human umbilical vein endothelial cell; IHR, intermittent hypoxia/reoxygenation.

and IL-6, were markedly enhanced by IHR compared with the control group (Fig. 3). Propofol at 50 or 100 μM significantly inhibited the increase of mRNA expression of TNF-α, IL-1β and IL-6 in the HUVECs exposed to IHR.

Effects of propofol on p38 MAPK. It was hypothesized that propofol attenuates the IHR-induced activation of NF-κB and its downstream proinflammatory cytokines by inhibiting p38 MAPK signaling. To test this hypothesis, the protein levels

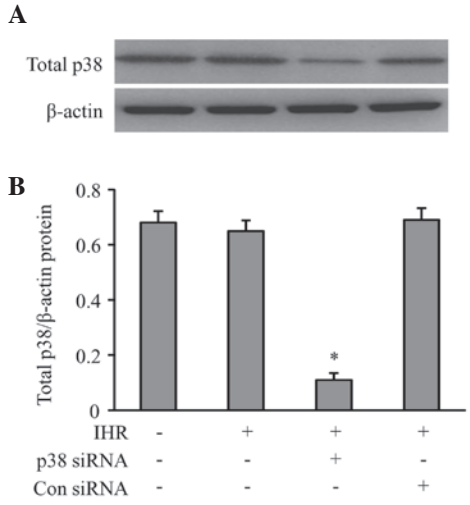

Figure 5. Effect of p38 siRNA transfection on total p38 MAPK expression. p38 siRNA, but not the control siRNA, effectively produced a knockdown of p38 MAPK by 84% in the HUVECs. The values are presented as the mean ± standard error (n=4 for each group). *P<0.05 vs. other three groups. Con, control; MAPK, mitogen-activated protein kinase; HUVEC, human umbilical vein endothelial cell.

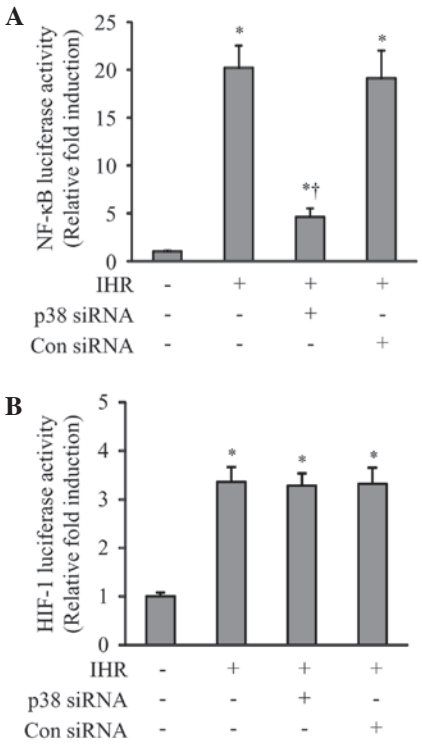

Figure 6. Effects of p38 siRNA on the activation of NF-κB and HIF-1 as measured by a luciferase reporter assay. Knockdown of p38 MAPK resulted in a significant reduction in the IHR-induced activation of (A) NF-κB, but not (B) HIF-1 in the HUVECs. The values are presented as the mean ± standard error (n=3-5 for each group). *P<0.05 vs. cells without any treatment; †P<0.05 vs. IHR alone or IHR + Con siRNA. Con, control; NF-κB, nuclear factor-κB; HIF-1, hypoxia-inducible factor-1; MAPK, mitogen-activated protein kinase; IHR, intermittent hypoxia/reoxygenation; HUVEC, human umbilical vein endothelial cell.

of total and phosphorylated p38 MAPK were determined by western blot analysis (Fig. 4). Compared with the control group, IHR increased the protein levels of phosphorylated p38 MAPK (Fig. 4B). Propofol at 25 and 50 μM dose-dependently reduced the protein levels of phosphorylated p38 MAPK in the HUVECs exposed to IHR, and high-dose propofol (100 μM) had no further effect on the phosphorylation of p38 MAPK. By contrast, the protein levels of total p38 MAPK were not affected by either IHR or propofol among the groups (Fig. 4C).

Effects of p38 siRNA on IHR-induced NF-κB and HIF-1 activity. To further confirm that propofol selectively reduces the NF-κB activity through inhibition of p38 MAPK signaling, the effects of p38 MAPK knockdown with siRNA were examined on the IHR-induced NF-κB and HIF-1 activity. Compared with the control siRNA, p38 siRNA effectively produced a knockdown of p38 MAPK by 84% in the HUVECs (Fig. 5), which was accompanied by a significant reduction in the IHR-induced activation of NF-κB (Fig. 6A), but not HIF-1 (Fig. 6B).

Discussion

The novel findings of the present study are summarized as follows: i) Propofol selectively inhibited the activation of NF-κB, but not HIF-1, in the HUVECs during IHR; ii) the reduced activation of NF-κB by propofol was accompanied by decreases in the levels of proinflammatory cytokines; and iii) the inhibitory effect of propofol on IHR-induced NF-κB activity was likely be based on the suppression of the p38 MAPK signaling pathway.

IHR is a hallmark feature of OSA and is associated with atherosclerosis, which is a chronic inflammatory disease involving a plethora of cell types and multiple pathological processes (16,17). Atherogenesis is triggered by vascular endothelial dysfunction that is characterized by a pro-inflammatory state of the endothelium (18,19), leading to endothelial apoptosis (19,20). NF-κB is a well-known redox-sensitive transcription factor involved in numerous pathological conditions, including inflammatory processes and cell apoptosis. In resting cells, NF-κB is predominantly localized in the cytoplasm in a complex with I-κB, which undergoes phosphorylation, ubiquitination and degradation upon stimulation, leading to the translocation of NF-κB into the nucleus followed by transcription of a battery of genes (21). Increasing evidence suggests that IHR may induce the activation of NF-κB and the release of proinflammatory cytokines. In addition to NF-κB, HIF-1, a transcription factor that is essential for regulating oxygen homeostasis, also regulates the expression of target genes, including proinflammatory cytokines (22). HIF-1 is a heterodimer composed of an oxygen-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit. Under hypoxic conditions, HIF-1α accumulates, translocates into the nucleus and determines the activity of HIF-1, which promotes the production of inflammatory cytokines. HIF-1 activity has been shown to be regulated through the NF-κB pathway (22). In the present study, 64 cycles of IHR significantly induced activation of NF-κB and HIF-1 in the HUVECs, accompanied by increased mRNA expression of TNF-α, IL-1β and IL-6. These results were consistent with previous findings *in vivo* and *in vitro*, demonstrating that NF-κB and HIF-1 are activated or produced in response to IHR and contribute to the expression

of proinflammatory cytokines (11,23). Thus, inhibition of the proinflammatory cytokines by suppressing NF-κB or HIF-1 activity in the vascular endothelium may be crucial to prevent atherosclerosis in patients with OSA.

The anti-inflammatory effects of propofol, an intravenous general anesthetic agent, have attracted attention in the studies of multiple diseases associated with inflammation. An *in vitro* study has demonstrated that propofol post-conditioning inhibits the activation of NF-κB induced by hypoxia/reoxygenation and protects cardiomyocytes against apoptosis (15). Other studies reported that propofol suppresses the activation of HIF-1 induced by lipopolysaccharides or hypoxia in macrophages (24), alveolar epithelial cells (25) and lung epithelial cells (22). However, the effects of propofol on NF-κB and HIF-1 activity in vascular endothelial cells subjected to IHR have not been previously assessed. In the present study, using HUVECs, propofol was demonstrated to dose-dependently inhibit IHR-induced NF-κB activity by suppressing the phosphorylation of I-κBa. However, propofol did not change the HIF-1 activity. In addition, the reduced NF-κB activity caused by propofol was accompanied by decreased mRNA expression of proinflammatory cytokines. These observations indicate that the anti-inflammatory effects of propofol in HUVECs during IHR are mainly due to the inhibition of the NF-κB pathway, which may have a dominant role over the HIF-1 pathway in regulating proinflammatory cytokines in the vascular endothelial cells. A previous study revealed that inhibition of the NF-κB pathway resulted in reduced HIF-1 activity in mouse embryonic stem cells, indicating that HIF-1 is downstream of the NF-κB pathway (26). Furthermore, propofol, at a similar dose range to that used in the present study, was reported to inhibit NF-κB and HIF-1 activity induced by lipopolysaccharides in lung epithelial cells (22). The discrepancy between the current data and previous findings may be due to the different cell types or methods used for studying the activation of NF-κB and HIF-1.

p38 MAPK is critical for the production of NF-κB-dependent proinflammatory cytokines (27,28). A previous study demonstrated that IHR activated NF-κB via activation of the p38 MAPK signaling pathway (7). The hypothesis that propofol may reduce IHR-induced NF-κB activity in HUVECs by suppressing p38 MAPK activity was tested. The data of the present study revealed that the phosphorylation of p38 MAPK was dose-dependently inhibited by propofol in a similar manner to the reduction in NF-κB activity. Furthermore, the knockdown of p38 MAPK with p38 siRNA led to a significant reduction in IHR-induced NF-κB activity. Together, these results indicate that the reduced NF-κB activity caused by propofol acts through the inhibition of the p38 MAPK signaling pathway in HUVECs exposed to IHR. Indeed, propofol has been identified to attenuate the lipopolysaccharide-induced production of proinflammatory cytokines and monocyte chemotactic protein-1 by inhibiting the phosphorylation of p38 MAPK in human THP-1 cells (14).

In conclusion, the present study demonstrated that propofol attenuates the IHR-induced activation of NF-κB, but not HIF-1, in vascular endothelial cells, and that these beneficial effects are possibly based on the inhibition of the p38 MAPK signaling pathway. Propofol may have the potential to prevent atherosclerosis in patients with OSA by inhibiting NF-κB-mediated inflammation in the vascular endothelium.

Acknowledgements

The present study was supported by the Shandong Provincial Natural Science Foundation of China (no. ZR2010HM120).

References

1. Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM and Mohsenin V: Obstructive sleep apnea as a risk factor for stroke and death. N Engl J Med 353: 2034-2041, 2005.
2. Quan SF, Gersh BJ; National Center on Sleep Disorders Research; National Heart, Lung, and Blood Institute: Cardiovascular consequences of sleep-disordered breathing: past, present and future: report of a workshop from the National Center on Sleep Disorders Research and the National Heart, Lung, and Blood Institute. Circulation 109: 951-957, 2004.
3. Fang G, Song D, Ye X, Mao SZ, Liu G and Liu SF: Chronic intermittent hypoxia exposure induces atherosclerosis in ApoE knockout mice: role of NF-κB p50. Am J Pathol 181: 1530-1539, 2012.
4. Lan L, Tao J, Chen A, *et al*: Electroacupuncture exerts anti-inflammatory effects in cerebral ischemia-reperfusion injured rats via suppression of the TLR4/NF-κB pathway. Int J Mol Med 31: 75-80, 2013.
5. Tang Z, Jiang L, Peng J, *et al*: PCSK9 siRNA suppresses the inflammatory response induced by oxLDL through inhibition of NF-κB activation in THP-1-derived macrophages. Int J Mol Med 30: 931-938, 2012.
6. Abe J: Role of PKCs and NF-kappaB activation in myocardial inflammation: enemy or ally? J Mol Cell Cardiol 43: 404-408, 2007.
7. Ryan S, McNicholas WT and Taylor CT: A critical role for p38 map kinase in NF-kappaB signaling during intermittent hypoxia/reoxygenation. Biochem Biophys Res Commun 355: 728-733, 2007.
8. Han Q, Yeung SC, Ip MS and Mak JC: Intermittent hypoxia-induced NF-κB and HO-1 regulation in human endothelial EA.hy926 cells. Cell Biochem Biophys 66: 431-441, 2013.
9. Song D, Fang G, Mao SZ, *et al*: Chronic intermittent hypoxia induces atherosclerosis by NF-κB-dependent mechanisms. Biochim Biophys Acta 1822: 1650-1659, 2012.
10. Htoo AK, Greenberg H, Tongia S, *et al*: Activation of nuclear factor kappaB in obstructive sleep apnea: a pathway leading to systemic inflammation. Sleep Breath 10: 43-50, 2006.
11. Ryan S, Taylor CT and McNicholas WT: Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. Circulation 112: 2660-2667, 2005.
12. Ryan S, Taylor CT and McNicholas WT: Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnoea syndrome? Thorax 64: 631-636, 2009.
13. Marik PE: Propofol: an immunomodulating agent. Pharmacotherapy 25: 28S-33S, 2005.
14. Tang J, Chen X, Tu W, *et al*: Propofol inhibits the activation of p38 through up-regulating the expression of annexin A1 to exert its anti-inflammation effect. PLoS One 6: e27890, 2011.
15. Li H, Tan J, Zou Z, Huang CG and Shi XY: Propofol post-conditioning protects against cardiomyocyte apoptosis in hypoxia/reoxygenation injury by suppressing nuclear factor-kappa B translocation via extracellular signal-regulated kinase mitogen-activated protein kinase pathway. Eur J Anaesthesiol 28: 525-534, 2011.
16. Drager LF, Polotsky VY and Lorenzi-Filho G: Obstructive sleep apnea: an emerging risk factor for atherosclerosis. Chest 140: 534-542, 2011.
17. Arnaud C, Dematteis M, Pepin JL, Baguet JP and Lévy P: Obstructive sleep apnea, immuno-inflammation, and atherosclerosis. Semin Immunopathol 31: 113-125, 2009.
18. Endemann DH and Schiffrin EL: Endothelial dysfunction. J Am Soc Nephrol 15: 1983-1992, 2004.
19. Kutuk O and Basaga H: Bcl-2 protein family: implications in vascular apoptosis and atherosclerosis. Apoptosis 11: 1661-1675, 2006.
20. Martinet W and Kockx MM: Apoptosis in atherosclerosis: focus on oxidized lipids and inflammation. Curr Opin Lipidol 12: 535-541, 2001.

21. Thurberg BL and Collins T: The nuclear factor-kappa B/inhibitor of kappa B autoregulatory system and atherosclerosis. Curr Opin Lipidol 9: 387-396, 1998.
22. Yeh CH, Cho W, So EC, *et al*: Propofol inhibits lipopolysaccharide-induced lung epithelial cell injury by reducing hypoxia-inducible factor-1alpha expression. Br J Anaesth 106: 590-599, 2011.
23. Li S, Qian XH, Zhou W, *et al*: Time-dependent inflammatory factor production and NFκB activation in a rodent model of intermittent hypoxia. Swiss Med Wkly 141: w13309, 2011.
24. Tanaka T, Takabuchi S, Nishi K, *et al*: The intravenous anesthetic propofol inhibits lipopolysaccharide-induced hypoxia-inducible factor 1 activation and suppresses the glucose metabolism in macrophages. J Anesth 24: 54-60, 2010.
25. He XY, Shi XY, Yuan HB, Xu HT, Li YK and Zou Z: Propofol attenuates hypoxia-induced apoptosis in alveolar epithelial type II cells through down-regulating hypoxia-inducible factor-1α. Injury 43: 279-283, 2012.
26. Lee SH, Lee YJ and Han HJ: Effect of arachidonic acid on hypoxia-induced IL-6 production in mouse ES cells: Involvement of MAPKs, NF-kappaB, and HIF-1alpha. J Cell Physiol 222: 574-585, 2010.
27. Kumar S, Boehm J and Lee JC: p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. Nat Rev Drug Discov 2: 717-726, 2003.
28. Karin M: Inflammation-activated protein kinases as targets for drug development. Proc Am Thorac Soc 2: 386-390, 2005.