Vascular Pharmacology 73 (2015) 149–157

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Propofol ameliorates endothelial inflammation induced by hypoxia/reoxygenation in human umbilical vein endothelial cells: Role of phosphatase A2

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ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 21 April 2015
Accepted 7 June 2015
Available online 10 June 2015

Keywords:
Hypoxia/reoxygenation
Propofol
Endothelial

ABSTRACT

Hypoxia/reoxygenation (H/R) induces endothelial inflammation with augmentation of endothelial adhesion molecules over-expression. Propofol was reported to attenuate endothelial adhesion molecule expression in some situations. Here, we examined the molecular mechanism for how propofol restored H/R-mediated up-regulation of endothelial adhesion molecules in human umbilical vein endothelial cells (HUVECs). Compared with the control group, H/R up-regulated expression of Pin-1 and PP2A, increased $p66^{Shc}$–Ser^{36} phosphorylation, induced $p66^{Shc}$ mitochondrial translocation, O_2^- accumulation and NF-κB activation, and decreased eNOS–Ser^{1177} phosphorylation and nitric oxide (NO) production, thus up-regulating expression of endothelial adhesion molecules and increasing mononuclear-endothelial interaction. More importantly, except that propofol had no effect on H/R-induced $p66^{Shc}$–Ser^{36} phosphorylation, most of H/R-mediated changes were alleviated by propofol, resulting in the reduction of endothelial adhesion molecules expression and mononuclear-endothelial adhesion. Moreover, we demonstrated the protective effect of propofol on H/R-induced endothelial inflammation was similar to that of calyculin A, an inhibitor of PP2A. In contrast, FTY720, an activator of PP2A, antagonized the effect of propofol. Our data indicated that propofol down-regulated PP2A expression, leading to reduced dephosphorylation of $p66^{Shc}$–Ser^{36} and eNOS–Ser^{1177}, which is associated with ROS accumulation and NO reduction, resulting in inhibition of endothelial adhesion molecule expression and mononuclear-endothelial interaction.

1. Introduction

Hypoxia/reoxygenation (H/R) in an *in vitro* situation mimics the ischemia/reperfusion (I/R) model *in vivo*. It leads to the accumulation of inflammatory cytokines [1,2] and activation of inflammatory signaling pathways in endothelial cells [3]. Endothelial inflammation consists of the augmentation of endothelial adhesion molecule expression, such as intercellular adhesion molecule 1(ICAM-1) and endothelial selectin (E-selectin). The increased expression of endothelial adhesion molecules induces mononuclear-endothelial adhesion, which leads to endothelial injury [4].

The nuclear factor kappa B (NF-κB) signal pathway was reported to be involved in H/R-induced endothelial adhesion molecules expression [5]. Previous data have indicated that reactive oxygen species (ROS) could activate NF-κB, which plays an important role in ROS-mediated endothelial injury due to H/R [6]. Mitochondrion is the major organelle where ROS is generated [7,8]. Furthermore, the $p66^{Shc}$ adaptor protein is important in regulating mitochondrial ROS generation [9–11]. The adjustment of $p66^{Shc}$ function is a complex process. First, $p66^{Shc}$ is phosphorylated at the site of Ser^{36}, then Ser^{36}–phosphorylated $p66^{Shc}$ is isomerized with a prolylisomerase Pin1 and dephosphorylated by the phosphatase A2 (PP2A) [12]. Only isomerized and dephosphorylated $p66^{Shc}$ could translocate to the mitochondria and subsequently lead to ROS generation [13].

It was also reported that reduced nitric oxide (NO) production from vascular endothelium induced endothelial adhesion molecule expression and leukocyte-endothelium adhesion [14,15], resulting in endothelial inflammation. Moreover, improvement of NO production could protect against H/R-induced endothelial injury [16]. And this effect was reversed by L-NANE, an endothelial NO synthase (eNOS) inhibitor [16].

Propofol (2, 6-diisopropylphenol) is a widely used intravenous anesthetic agent. It was reported that propofol could inhibit the expression of endothelial adhesion molecules in H/R-treated endothelial cells [17]. However, the mechanism by which propofol protects against H/R-induced adhesion molecules expression is still elusive. In the present study, we examined whether and how propofol protects HUVECs against H/R-induced endothelial adhesion molecules expression.

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http://dx.doi.org/10.1016/j.vph.2015.06.002
1537-1891/

2. Methods

2.1. Cell culture and reagents

HUVECs (Clonetics) were incubated in Dulbecco's Modified Eagle's Medium (DMEM) and 10% fetal bovine serum in incubator containing 5% CO_2 at 37 °C. Cells were sub-cultured when reaching around 90% confluence. The fourth passage of HUVECs was employed in the present study.

Propofol (sigma), calyculin A (sigma) and FTY720 (sigma) were dissolved in dimethyl sulfoxide (DMSO) (sigma). The final concentration of DMSO was regulated to 0.01% for each medium to minimize any potential toxicity or non-specific effects.

2.2. Study design

During general anesthesia, clinic plasma concentrations of propofol range from 5 to 50 μM [18]. To mimic the situation in an *in vivo* model, HUVECs were pre-incubated with various concentrations (1, 5, and 25 μM) of propofol for 30 min, followed by H/R treatment. The intervention groups of HUVECs were incubated with pre-equilibrated (1% O_2) culture medium in a hypoxic incubator at 1% O_2, 5% CO_2 and 37 °C for 1 h, followed by 2 h reoxygenation with medium upon reoxygenation.

2.3. Western blot analysis

Whole-cell extracts were acquired by using of cell lysis buffer (Cell Signaling Technology, Danvers, MA). Mitochondrial extracts, cytosol extracts and nuclear extracts were acquired separately with the use of mitochondrial Extract Kit (Shanghai Shengong Bioengineering Institute, Shanghai City, PR China). Equal amount of protein extracted from different portion was loaded and separated by 8% or 10% SDS-PAGE and thereafter transferred to Polyvinylidene Fluoride (PVDF) membranes. After being blocked in 5% fat-free milk, the membranes were incubated with primary antibodies at 4 °C overnight. The primary antibodies were antibody against β-actin (Santa Cruz Biotechnology), cytochrome c oxidase IV (COX IV) (Santa Cruz Biotechnology), $p66^{Shc}$ (epitomics), p-$p66^{Shc}$–Ser^{36} (epitomics), ICAM-1(cell signal), E-Selectin (Santa Cruz Biotechnology), NF-κB (cell signal), eNOS (Santa Cruz Biotechnology), p-eNOS-Ser^{1177} (cell signal), pin-1 (epitomics) and PP2A (cell signal). Thereafter, the primary antibodies were washed in TBST for 3 times, and the membranes were incubated with secondary antibodies for 1 h at room temperature. Subsequently, the membranes were washed three times with TBST and detected by the ECL system. The respective densities of the protein bands were analyzed by Scan-gel-it software. In the present study, β-actin was used as loading control in whole-cell extracts and cytosol extracts, COX IV was used as loading control in mitochondrial extracts, while Histone H3 was used as loading control in nuclear extracts. Also, the data were interpreted as the ratio of specific protein expression to beta-actin expression, COX IV or Histone H3, respectively.

2.4. Superoxide anion (O_2^-) accumulation assay

O_2^- accumulation was detected by the reduction of ferricytochrome c assay as described previously [19]. Briefly, HUVECs (3000 cells/100 ul) were added to all wells in the 96-multiwell plate and cultured at 37 °C.

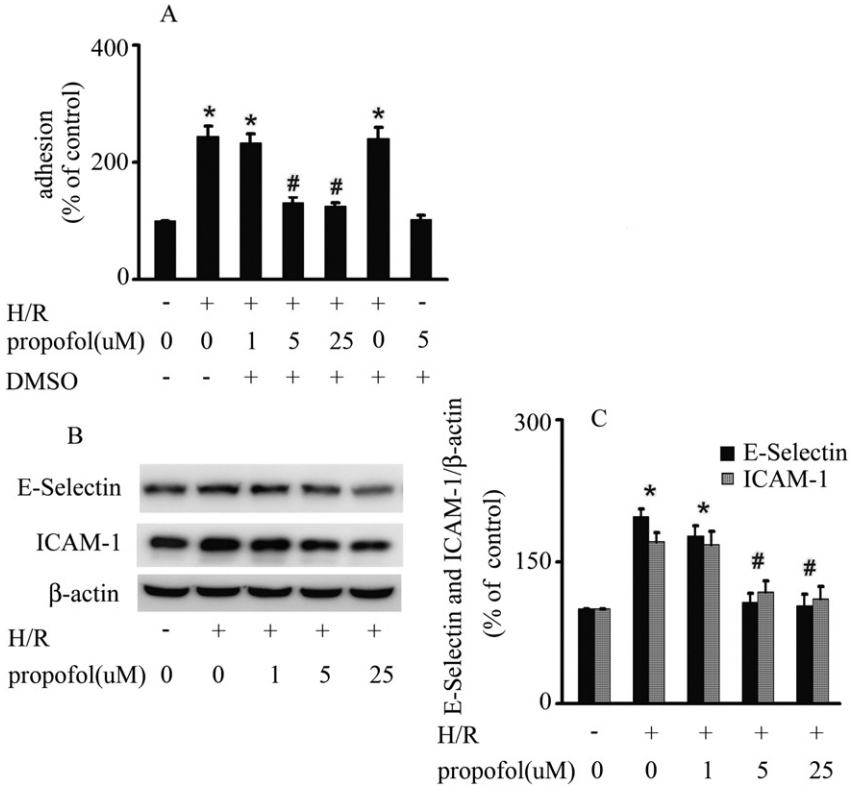

Fig. 1. Effects of propofol on H/R-mediated augment of mononuclear-endothelial adhesion and increase of endothelial adhesion molecules expression. Cells were pre-incubated with propofol (1, 5, 25 μM) for 30 min, then incubated in a hypoxic incubator at 1% O_2, 5% CO_2 at 37 °C for 1 h, followed by 2 h re-oxygenation. Cells were cultured in DMEM without H/R treatment as a control. (A) Compared with control group, H/R treatment induced mononuclear-endothelial interaction which was reversed by propofol in a concentration-dependent manner. Propofol solvent dimethyl sulfoxide (DMSO) did not affect H/R-mediated augment of mononuclear-endothelial adhesion. Also pre-incubation of cells with 5 μM propofol for 30 min had no effect on base mononuclear-endothelial adhesion. (B, C) Western blot and densitometric quantification of endothelial adhesion molecule expression. (*$p < 0.05$ *vs.* control group; #$p < 0.05$ *vs.* H/R treatment, $n = 5$ independent experiments. Data are shown as mean ± SD.).