Vascular Pharmacology 61 (2014) 72–79

Contents lists available at ScienceDirect

Vascular Pharmacology

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

Propofol protects human umbilical vein endothelial cells from cisplatin-induced injury

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ARTICLE INFO

Article history:
Received 13 November 2013
Received in revised form 24 March 2014
Accepted 2 April 2014
Available online 13 April 2014

Keywords:
Cisplatin
Propofol
eNOS
ICAM-1
Endothelium

ABSTRACT

The anticancer drug cisplatin can up-regulate endothelial adhesion molecule expression, and trigger vascular endothelial injury. Propofol, an intravenous anesthetic, can inhibit endothelial adhesion molecule expression in some situations. Here, we explored whether and how propofol improved cisplatin-induced up-regulation of endothelial adhesion molecules in human umbilical vein endothelial cells. Compared with control group, cisplatin reduced endothelial nitric oxide synthase dimer/monomer ratio, activated protein kinase C and enhanced endothelial nitric oxide synthase-Thr^{495} phosphorylation, decreased nitric oxide production, augmented intercellular adhesion molecule 1 expression and monocyte-endothelial adhesion. These cisplatin-mediated effects were attenuated by propofol treatment. Nω-Nitro-L-arginine methyl ester hydrochloride, a nitric oxide synthase inhibitor, inhibited the effect of propofol on cisplatin-induced intercellular adhesion molecule 1 expression. Propofol improved cisplatin-mediated tetrahydrobiopterin reduction and nitrotyrosine overexpression. Compared with control group, cisplatin and PMA, a protein kinase C activator, both increased endothelial nitric oxide synthase-Thr^{495} phosphorylation, while propofol and GFX, a protein kinase C inhibitor, both decreased cisplatin-induced endothelial nitric oxide synthase-Thr^{495} phosphorylation. Our data indicated that propofol, via reducing cisplatin-induced endothelial nitric oxide synthase uncoupling and endothelial nitric oxide synthase-Thr^{495} phosphorylation, restored nitric oxide production, intercellular adhesion molecule 1 expression and monocyte-endothelial interaction.

1. Introduction

Cisplatin is a widely used chemotherapy agent in anti-cancer treatment with significant benefits in many patients. However, some safety concerns have been reported, such as nephrotoxicity [1–3], ototoxicity [4–6], neurotoxicity [7–10], and increased incidence of thrombosis [11]. These concerns have limited it from benefiting more patients in clinical practice. Previous studies have demonstrated that part of the cisplatin-mediated side effects may be attributed to potential vascular endothelium injury [12–16]. Consistent with this, recent studies reported that cisplatin-based chemotherapy was associated with higher incidence of atherosclerotic disease, coronary artery disease, and myocardial infarction [17]. The underlying mechanisms may be explained by cisplatin-induced up-regulation of adhesion molecules and leukocyte-endothelial adhesion [18], which in turn triggers endothelial dysfunction and ischemic injury of tissues and organs.

Nitric oxide (NO) is an important signaling chemokine in vascular homeostasis. In vascular endothelium, NO is mainly produced by endothelial NO synthase (eNOS), and is a potent vasodilator [19] with the property of anti-platelet [20], anti-leukocyte adhesion [21], anti-inflammation, and cyto-protection [22]. Disruption of NO balance is a key feature of endothelial dysfunction in various vascular diseases. Large amount of evidence obtained from animal and human studies indicated that imbalance of synthesis and bioavailability of NO is involved in many cardiovascular diseases [23]. Previous studies have indicated that the reduction of NO production is associated with severe endothelial injury in in vitro and in animal studies [24–26]. Further, such endothelial injury could be improved by the restoration of NO production [24,26]. Nevertheless, the molecular mechanism is far from clear. It was reported that impaired NO production up-regulates expression of adhesion molecules and enhances the interaction between leukocytes and endothelium [27,28], thus increasing the incidence of endothelial injury [29]. A previous study has demonstrated that cisplatin induced endothelial dysfunction by attenuating NO production in human umbilical vein endothelial cells (HUVECs) [30]. Interventions which target monocyte-endothelial interaction could attenuate endothelial injury [31].

Recently, more and more patients who suffer from cancers are receiving cisplatin-based chemotherapy combined with surgical resection.

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http://dx.doi.org/10.1016/j.vph.2014.04.001
1537-1891/

Cisplatin-induced endothelial injury may be exaggerated perioperatively. These patients usually need general anesthesia. Propofol (2,6-diisopropylphenol) is a widely used intravenous anesthetic agent with a satisfactory safety profile. Previous studies indicated that propofol could protect vascular endothelial injury via its effects of anti-endothelial dysfunction [32] and anti-inflammation [33] in many situations. In this study, we therefore investigated whether propofol could protect vascular endothelium, which was exposed to cisplatin. If so, we could alleviate these cisplatin-induced noxious effects by choosing appropriate agents. Further, we investigated the underlying mechanisms.

2. Materials and methods

2.1. Cell culture

HUVECs (Clonetics; Lonza, Basel, Switzerland) were cultured in DMEM with 5 mM glucose and 10% fetal bovine serum in incubator containing 5% CO_2 at 37 °C. Cells were sub-cultured when reaching 90% confluence. The fourth passage of HUVECs was used in the present study.

2.2. Study design

HUVECs were treated with different concentrations of cisplatin (Sigma, St. Louis, MO) (0.1, 0.5, 1.0, and 5.0 μg/ml) for different time courses (6, 12, 24 and 36 h). By measuring monocyte-endothelial adhesion, we determined the appropriate cisplatin treatment condition with maximal effect on monocyte-endothelial adhesion. During general anesthesia, plasma concentrations of propofol (Sigma, St. Louis, MO) range from 5 to 50 μM [34]. To mimic in vivo situation, after cisplatin treatment, HUVECs were incubated with different concentrations (5, 10, 20, and 40 μM) of propofol for 2 h. The optimal concentration of propofol with significant inhibitory effects on monocyte-endothelial adhesion was determined. These treatment conditions were used in the following studies in which HUVECs were cultured and divided into four groups to examine the underlying signaling pathways. Group 1: HUVECs were cultured in DMEM as control; Group 2: HUVECs were treated with 20 μM propofol for 2 h; Group 3: HUVECs were treated with 1 μg/ml cisplatin for 24 h; Group 4: HUVECs were treated with 1 μg/ml cisplatin for 22 h and co-incubated with 20 μM propofol for the last 2 h.

2.3. Isolation and adhesion of monocytes to HUVECs

Human monocyte isolation was accomplished with the use of Histopaque-1077 (Sigma, St. Louis, MO) according to the manufacturer's instructions as described previously [33]. Briefly, 8 ml heparinized blood from volunteers was layered onto 8 ml Histopaque-1077. The monocytes were harvested after the samples were centrifuged at 400 g for 30 min. Isolated monocytes were washed with PBS, and re-suspended in the DMEM, and then added to the HUVECs. After being cultured at 37 °C for 30 min, cells were washed with PBS, and observed under a phase-contrast microscope. Adherent cells were counted in 10 different fields from five separate culture dishes. The data were expressed as folds increased compared with control group. The present study was performed in accordance with the declaration of Helsinki.

2.4. Western blot analysis

Equal amount (60 μg) of protein extracted from different groups of HUVECs was separated by 6% SDS-PAGE and transferred to nitrocellulose membranes. To study eNOS dimer/monomer expression, low-temperature SDS-PAGE was performed as described previously [24]. After being blocked in 5% skim milk, the membranes were incubated with an antibody at 4 °C for overnight. The primary antibodies were monoclonal antibody against β-actin (Santa Cruz Biotechnology, Santa Cruz, CA), and polyclonal antibody against intercellular adhesion molecule 1 (ICAM-1) (Cell Signaling Technology, Danvers, MA), eNOS (Santa Cruz Biotechnology, Santa Cruz, CA), p-eNOS-Thr^{495} (Santa Cruz Biotechnology, Santa Cruz, CA), p-eNOS-Ser^{1177} (Cell Signaling Technology, Danvers, MA), and nitrotyrosine (Abcam, Cambridge, UK). Thereafter, the primary antibodies were washed away, and the membranes were incubated with secondary antibodies for 1 h at room temperature. Subsequently, the membranes were washed with TBST for three times, and detected by the ECL system. The respective densities of the protein bands were analyzed by Scan-gel-it software. Peroxynitrite reacts with protein tyrosine residues to form nitrotyrosine, so this can be used as a marker of peroxynitrite production. In the present study, β-actin was used as loading control. The data were expressed as the ratio of specific protein expression and beta-actin expression.

2.5. NO production assay

NO production was determined by a nitrate reductase assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing City, P. R. China) according to the manufacturer's instructions. Briefly, cell culture medium, reagent 1 and reagent 2 were mixed and kept at 37 °C for 60 min. Then, reagent 3 and reagent 4 were added, mixed and kept at room temperature for 40 min. The supernatant was harvested after the samples were centrifuged at 3500 rpm for 10 min. Then, chromogenic agent was added and results were detected at 550 nM spectrophotometrically. The data were expressed as the percentage of the control group.

2.6. Total biopterin and tetrahydrobiopterin (BH_4) level determination

Biopterin levels were detected as described previously [32]. In brief, HUVECs were lysed in pre-cold extract buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM DTT). After protein concentration was determined, a 1:1 mixture of 1.5 M $HClO_4$ and 2 M H_3PO_4 was used to remove protein. Total biopterin (BH_4, dihydrobiopterin, biopterin) was measured after acid oxidation, while dihydrobiopterin and biopterin were measured after base oxidation. Biopterin was measured by liquid chromatography-mass spectrometry. The BH_4 level was counted by subtracting dihydrobiopterin and biopterin from total biopterin. The results were shown as the percentage of the control group.

2.7. Protein kinase C (PKC) activity assay

PKC activity was measured with SignaTECT PKC assay system (Promega, Madison, WI) according to the manufacturer's instructions as described previously [33]. Briefly, membrane extracts, reaction buffer and [γ-^{32}P] ATP were mixed and kept at 30 °C for 10 min. PKC phosphorylation was measured by detecting the radioactivity. PKC activity was determined by subtracting the enzymatic activity without phospholipids from the enzymatic activity with phospholipids. The data were expressed as fold increase compared with control group.

2.8. Superoxide anion (O_2^-) accumulation assay

O_2^- accumulation was measured by the reduction of ferricytochrome c assay as described previously [32]. Briefly, cells were washed and cultured with Krebs-HEPES buffer containing 20 μM ferricytochrome c (Sigma, St. Louis, MO) in the presence or in the absence of superoxide dismutase (Sigma, St. Louis, MO). The absorbance was read at 550 nm spectrophotometrically. Reduction of ferricytochrome c in the presence of superoxide dismutase was subtracted from the data in the absence of superoxide dismutase. Arbitrary unit was used as the unit for absorbance difference. Then we counted the cell number, and expressed the data as arbitrary unit/10^6 cells.