Baicalein attenuates vinorelbine-induced vascular endothelial cell injury and chemotherapeutic phlebitis in rabbits

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ABSTRACT

Chemotherapy is one of the major strategies for cancer treatment. Several antineoplastic drugs including vinorelbine (VRB) are commonly intravenously infused and liable to cause serious phlebitis. The therapeutic drugs for preventing this complication are limited. In this study, the mechanism of baicalein (BCN) was investigated on VRB-induced phlebitis in vivo and vascular endothelial cell injury in vitro. Treatment with BCN obviously attenuated vascular endothelial cell loss, edema, inflammatory cell infiltration and blood clots, and reduced the serum levels of TNF-α, IL-1β, IL-6 and ICAM-1 in the rabbit model of phlebitis induced by intravenous injection of VRB compared with vehicle. Further tests in vitro demonstrated that BCN lessened VRB-induced endothelial cell apoptosis, decreased intracellular ROS levels, suppressed phosphorylation of p38 and eventually inhibited activation of NF-κB signaling pathway. And these effects could be reversed by p38 agonist P79350. These results suggested that BCN exerted the protective effects against VRB-induced endothelial disruption in the rabbit model of phlebitis via inhibition of intracellular ROS generation and inactivation of p38/NF-κB pathway, leading to the decreased production of pro-inflammatory cytokines. Thus, BCN could be used as a potential agent for the treatment of phlebitis.

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1. Introduction

Chemotherapy is one of the major strategies for cancer treatment. Given their short half-lives and low bioavailabilities, many chemotherapy drugs require repeated administration for patients to achieve therapeutic benefits. However, long-term repeated intravenous chemotherapy could cause several toxic effects, including nephrotoxicity, neurotoxicity, hepatotoxicity, myelosuppression, and superficial venous injury (Gustavsson et al., 2015; Ménard et al., 2008; Roness et al., 2014). Phlebitis is a common and acute toxic reaction in 70% of patients undergoing chemotherapy. Its occurrence is related to irritant antineoplastic drugs, catheter material, and inaccurate aseptic manipulation during infusion (Cicolini et al., 2014; Norton et al., 2013). As the sterile inflammation of a vein, phlebitis is accompanied by pain, erythema, edema, varicose veins, and thrombosis. Although phlebitis is a benign process, failure to take a medicine as prescribed can cause considerable distress and unnecessary disease progression, as well as significant disability in advanced patients (Ray-Barruel et al., 2014). Despite effective measures of intensive supportive care (such as sclerotherapy, thermal ablation, and surgical stripping), these alternatives require further improvement (Eberhardt and Raffetto, 2014; Spierring, 2014; van den Bos et al., 2011). Furthermore, few desired agents have been used in clinical treatment (dos Reis et al., 2009), and candidates for superficial venous disease remain seriously inadequate.

Baicalein (BCN) is a pluripotent flavonoid extracted from the root of Scutellaria baicalensis Georgi. It is clinically used for the treatment of enteritis, diarrhea, and dysentery, as well as paralysis caused by cerebrovascular disorders, due to its various pharmacological actions, such as antimicrobial, antiviral, anti-inflammatory, anticancer, hypolipidemic, antiatherogenic, antithrombotic, and immunoregulatory effects (Chen et al., 2014; Kim et al., 2014; Kwak et al., 2014; de Oliveira et al., 2015; Li-Weber, 2009). Recent studies have discovered that BCN potentially protects human umbilical vein endothelial cells (HUVECs) against ox-LDL- and H2O2-mediated oxidative injury. It can significantly decrease vascular permeability and monocyte adhesion, as well as inhibit the expression of cell adhesion molecules (CAMs), the production of reactive oxygen species (ROS), and the activation of nuclear factor (NF)-κB (Bertin et al., 2016; Kwak et al., 2014; Lee et al., 2015). Furthermore, BCN reverses multidrug resistance through glycolysis inhibition and DR5 induction in several cancer cells. When combined with other chemotherapy agents, BCN can exert enhanced synergistic anticancer
effects (Chen et al., 2015a; Meng et al., 2016; Taniguchi et al., 2008; Wang et al., 2015). Remarkably, BCN hardly causes cytotoxicity and mutagenesis in normal human cells, such as hematopoietic cells and hepatocytes (Taniguchi et al., 2008). These multiple properties may contribute to the potential benefit of BCN to patients suffering from superficial venous diseases. This study investigated the protective effects of BCN against vascular endothelial cell injury induced by vinorelbine (VRB), an irritant antineoplastic drug commonly used in clinical cancer treatment, in HUVECs and in a rabbit model of auricular phlebitis.

2. Materials and methods

2.1. Drugs

Vinorelbine (VRB) bitartrate injections (10 mg/vial) were purchased from HISUN pharmaceutical Co. Ltd., Zhejiang, China. Mucopolysaccharide polysulfate (MP) ointment (content 3%) was purchased from Germany Sankyo Pharmaceutical Co. Ltd., Germany. BCN (purity > 98.0%) and quercetin (purity > 97.3%) were purchased from Aladdin Co. Ltd., Shanghai, China. A vial of VRB was diluted with 5 ml of normal saline, while 0.5 g of BCN was dissolved with 2 ml of oil and then diluted with white vaseline to be a 2% BCN ointment.

2.2. Animal model

Male Specific Pathogen-Free Japanese white rabbits (weights: 1.8–2.2 kg), obtained from Zhejiang Animal Experimental Center, were housed in groups of five per standard cage, on 12 h light/dark cycle; and air temperature was maintained at 26 ± 1 °C. Experiments were implemented in accordance with Chinese animal welfare guidelines and were approved by the institutional ethics committee. All rabbits were fed in different cages taking drink freely.

The phlebitis model was prepared as previously described (Kohno et al., 2008). Fifty rabbits were randomly divided into five groups: the normal control group (NC), the model control group (MC), vehicle (atolinol and vaseline; same prescription used for preparing BCN ointment) control group (VC), MP-treated group (MPT), BCN-treated group (BCNT). Each group had ten rabbits. Except NC, other animals were intravenously injected with VRB (3.0 mg/kg body weight) via a peripheral vein of right ear once daily for two days; injection lasted for 30 min at 5 ml/kg/h. The rabbits in NC were challenged with the saline solution. Rabbits in MPT were administered 3% MP ointment in a dosage of 0.1 g/kg corresponding to 3 mg MP/kg (Hitosugi et al., 2004), while BCNT rabbits were administered 2% BCN ointment in a dosage of 0.1 g/kg corresponding to 2 mg BCN/kg (Kim et al., 2013). Ointments were smeared on the skin of phlebitis region (1.5 cm × 2.0 cm) once daily for 5 d, starting from 2 h after VRB first challenge. The saline was smeared on the same region of the rabbit ears in NC and MC.

2.3. Histopathology analysis

A part of ear tissue in each group was prepared routinely with the clinical, etiologic, anatomic, pathologic (CEAP) classification was used to assess histopathological changes as reported previously (Kohno et al., 2008).

2.4. Immunohistochemistry analysis

The expressions of phosphorylated p38 MAPK, total p38 MAPK, NF-κB p65 and iNOS (Abcam, UK) were detected by using corresponding immunohistochemistry kits (Genomeditech. Co. Ltd., Hangzhou, China). The optical density of the positive cells was analyzed by using the software Image Pro Plus (v 6.0). Five discontinuous histological views of each slice were observed under the microscope (DM2500, Leica) to see the pathological changes of the ear tissue.

2.5. Cell culture and cell viability assay

HUVEC were cultured in Dulbecco’s Modified Eagle Medium (DMEM; Gibco, USA) and supplemented with 10% of fetal bovine serum (Tianjing Bioproducts Co. Ltd., Deqing, China). Cells were random-dized into 7 groups: the normal control group (NC), the model control group (MC; treated with 10 μg/ml of VRB), the positive control group (treated with 20 μg/ml of quercetin) and four BCN-treated groups (treated with 5, 10, 20 and 40 μg/ml, respectively). The concentrations of BCN and quercetin were used in this study as reported previously (Yamada et al., 2010; Yang et al., 2015). BCN or quercetin was dissolved in dimethyl sulfoxide (DMSO) and then diluted into the culture medium. Except NC, cells in other groups were incubated for 1–48 h after co-treated with VRB (20 μg/ml), BCN (5–40 μg/ml) and/or p38 agonist P79350 (50 μM, final concentration). After added with 1 mg/ml MTT-containing medium, the cells were cultured for 4 h. Then, DMSO (100 μl) was added to solubilize formazan. The absorbance of samples was detected at 570 nm by the multifunctional Microplate Reader (MD, USA).

2.6. Measurement of intracellular reactive oxygen species (ROS) production

After treated with VRB (10 μg/ml) and BCN (5–20 μg/ml) for 48 h, cells were stained by the fluorescent dye DCFH-DA and then incubated for 30 min in the shade at 37 °C. The fluorescence images of cells were obtained by BX43 Olympus microscopy (excitation at 490 nm and emission at 530 nm). The samples were harvested and then the fluorescence intensity of DCFDA-stained cells was detected by flow cytometry (BD, USA).

2.7. Cell apoptosis analysis

The HUVEC cells were cultured in 6-well plates. After co-exposed to BCN and VRB for 48 h, cells were collected and then suspended in binding buffer. Finally, these samples were treated with annexin V-FITC and propidium iodide (PI) by using the apoptosis detection kit (YEASEN, USA), and then analyzed by flow cytometry (BD, USA).

2.8. Mitochondrial membrane potential (MMP) assay

After treated with BCN (5–20 μg/ml) for 48 h, the cells were stained with JC-1 dye (2 μmol/l) for 30 min at 37 °C. Red/green fluorescence values were analyzed by using the microscope (DM2500, Leica) and its own software.

2.9. Cytokine assay

The serum from the rabbit models was collected and then assayed by using the ELISA kits (Biotech Co. Ltd., Nanjing, China). Caspase-3 activity of cells was also assayed by using the colorimetical kits (R&D, USA).

2.10. Western blot analysis

Cells or tissue homogenate were collected and then lysed in RIPA buffer. The cell lysates were centrifuged at 12,000 rpm for 20 min at 4 °C and protein concentration of sample was determined by the BCA method. Protein preparations were subjected to 12.5% SDS-PAGE, and electrophoretically transferred onto PVDF membranes. The membranes were incubated overnight at 4 °C with an antibody reactive with phospho-p38 MAPK (p-p38), p38 MAPK, NF-κB p65, iNOS, cleaved caspase-9, cleaved caspase-3 and the control β-actin antibodies (Abcam, UK). After washed triple with 1% Tween 20-containing PBS, the blots were incubated with the horseradish peroxidase-conjugated secondary antibodies for another 1 h. The signals were detected by using Enhanced Chemiluminescence and the chemiluminescence detector (Bio-Rad, USA).
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