Carbohydrate Polymers 105 (2014) 20–25

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Inhibition of inflammatory injure by polysaccharides from *Bupleurum chinense* through antagonizing P-selectin

Haibin Tong [a,*], Dan Tian [a], Tianbao Li [d], Bo Wang [b], Guiquan Jiang [c], Xin Sun [a]

[a] *Life Science Research Center, Beihua University, Jilin 132013, China*
[b] *College of Chemistry and Biology, Beihua University, Jilin 132013, China*
[c] *College of Forestry, Beihua University, Jilin 132013, China*
[d] *Jiangmen Entry-exit Inspection and Quarantine Bureau, Jiangmen 529000, China*

ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 10 January 2014
Accepted 12 January 2014
Available online 21 January 2014

Keywords:
Bupleurum chinense
Polysaccharide
Acute inflammation
P-selectin
Neutrophil adhesion

ABSTRACT

P-selectin-mediated adhesion between endothelium and neutrophils is a crucial process leading to acute inflammatory injure. Thus, P-selectin has been considered as promising target for therapeutics of acute inflammatory-related diseases. In the present study, the water-soluble polysaccharides (BCPs) were isolated from *Bupleurum chinense*, and we evaluated their therapeutical effects on acute inflammatory injure and antagonistic function against P-selectin-mediated neutrophil adhesion. Our results showed that BCPs significantly impaired the leukocyte infiltration and relieve lung injury in LPS-induced acute pneumonia model. BCPs significantly blocked the binding of P-selectin to neutrophils and inhibited P-selectin-mediated neutrophils rolling along CHO-P cell monolayer. The result from *in vitro* protein binding assay showed a direct evidence indicating that BCPs-treatment significantly eliminated the interaction between rhP-Fc and its physiological ligand PSGL-1 at protein level. Together, these results provide a novel therapeutical strategy for amelioration of inflammation-related disease processes by polysaccharides from *B. chinense*.

1. Introduction

Most bacteria are detected and destroyed within hours of entering the host by the defense mechanism of acute inflammation known as innate immunity (Nathan, 2006). Polymorphonuclear neutrophils (PMNs) are the predominant cell type involved in the acute inflammation after infection. Since excessive inflammation is harmful to normal tissues, once the pathogen has been removed the acute inflammation response is terminated (Yoshikai, 2001). However, under some special pathological circumstances, the improper recruitment and activation of neutrophils during acute inflammatory response contribute to organ dysfunction, serious tissue injury, and inflammation-related diseases, and a poorly controlled acute inflammatory response even leads to death (Grommes & Soehnlein, 2011; Segel, Halterman, & Lichtman, 2011; Tanya, George, & Naotake, 2009). Thus, the restriction of neutrophil recruitment into tissue compartments has been considered as an effective strategy in amelioration of the acute inflammatory response-related injure process (Ulbrich, Eriksson, & Lindbom, 2003).

The recruitment of neutrophils from blood to infected tissue is an important step of acute inflammation response, which is a dynamic and coordinated multi-step process regulated by a variety of adhesion molecules (Kelly, Hwang, & Kubes, 2007; Kubes & Ward, 2006; Simon & Green, 2005). P-selectin on activated endothelial cells, as a member of the selectin family of cell adhesion molecule, plays a key role in neutrophil recruitment during acute inflammatory response, and its main function is to regulate the initial attachment of neutrophil with the vessel endothelial cells (Geng, Chen, & Chou, 2004; Somers, Tang, Shaw, & Camphausen, 2000). Therefore, P-selectin has been considered as a promising therapeutic target for interfering and controlling acute inflammation-related pathological processes, and researchers focus on searching for high affinity glycoconjugate ligands from natural products for antagonists of P-selectin-mediated neutrophil initial recruitment (Barthel, Gavino, Descheny, & Dimitroff, 2007; Hackert, Büchler, & Werner, 2010; Woollard & Chin-Dusting, 2010). Among various natural carbohydrate compounds, polysaccharide extracted from medicinal herbs might prove to be one of the promising candidates in searching for high affinity natural products with antagonistic function against P-selectin (Fei et al., 2008; Tong et al., 2011).

In our previous study, we reported the physicochemical properties and structural characterization of the water-soluble polysaccharide from *B. chinense*, which is a heteropolysaccharide

* Corresponding author. Tel.: +86 432 64608351; fax: +86 432 64608350.
E-mail address: tonghaibin@gmail.com (H. Tong).

0144-8617/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2014.01.039

mainly composed of arabinose; galactose; glucose with a molar ratio of 2.1:2.5:1 (Sun et al., 2010). According to FT-IR, partial acid hydrolysis, periodate oxidation and Smith degradation, methylation and GC–MS analysis, we found this polysaccharide had a backbone of (1→5)-linked arabinose, (1→4)-linked galactose and (1→3)-linked galactose residues with occasionally branches at O-6, and the branches were composed of (1→4)-linked glucose, and terminated with galactose residues. In the present study, we further evaluated the effects of water-soluble polysaccharides from *B. chinense* on acute inflammation and antagonistic activity against P-selectin-mediated function by *in vivo* and *in vitro* model. Here, we identify a novel anti-P-selectin-mediated cell adhesion agent, and the previous finding and our present results provide theoretical support for those polysaccharides as anti-inflammatory drugs.

2. Materials and methods

2.1. Materials and chemicals

The water-soluble *B. chinense* polysaccharides (BCPs) were isolated and characterized as previously described (Tong et al., 2013). Recombinant human P-selectin/Fc chimera protein (rhP-Fc) and blocking mAb to P-selectin (9E1) were obtained from R&D Systems (Minneapolis, MN, USA). The monoclonal antibodies for P-selectin (P8G6) and PSGL-1 (PL1) were obtained from Santa Cruz Biotechnology. A non-blocking mAb to P-selectin (AC1.2) was purchased from BD PharMingen (Franklin Lakes, New Jersey, USA). Goat anti-human fluorescein-isothiocyanate (FITC)-labeled immunoglobulin G (IgG) and goat anti-mouse IgG were purchased from Jackson Immuno-Research Laboratories (West Grove, Pennsylvania, USA). The negative isotype controls, depending on the species and subclasses of the primary antibodies used, were obtained from Santa Cruz Biotechnology. Calcein acetoxymethylester (Calcein-AM) was purchased from Invitrogen. All other chemical reagents used were analytical grade.

CHO cells were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Science (Shanghai, China). CHO-P cells stably expressing human P-selectin were obtained by transfecting full-length human P-selectin vector into CHO cells. All cells were grown in IMDM (Gibco) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.2. Neutrophil isolation

Human neutrophils were freshly isolated from whole blood from healthy human volunteers using the density gradient technique (Filippi, Szczur, Harris, & Berclaz, 2007; Nauseef, 2007). Remaining erythrocytes were removed by hypotonic lysis. More that 95% of the cells isolated were neutrophils as assessed by Wright-Giemsa staining, and the viability was determined to be >95% by trypan blue exclusion test. The isolated neutrophils were kept on ice and used within 4 h.

2.3. LPS-induced acute pneumonia model and HE staining

Male BALB/c mice (18–22 g) were purchased from Animal Experimental Center of Jilin University. The mice were housed in plastic cages and kept under standardized conditions at a temperature of 22–24 °C, and 20% humidity with a 12 h light/dark cycle, and free access to tap water and food. They were allowed to acclimatize for 3 days before the experiments started. Mice were intravenously administrated saline solution with or without BCPs (50 and 200 mg/kg). 30 min after BCPs administration, LPS was administered intranasal for pulmonary delivery. The animals were suspended vertically. LPS (50 μg in 50 μl saline) was instilled into the nares. The mice were maintained in vertical orientation for 5 min to allow full penetration of the LPS into the lungs. 4 h after LPS treatment, the mice were anesthetized with isoflurane and the lungs were removed for further histologic analysis. Normal control mice were produced with the treatment of saline.

For HE staining, lung tissues were perfused with a fixative solution containing 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.0), and incubated overnight at 4 °C. After washing three times, the tissues were processed in preparation for paraffin embedding and cut into 5-μm-thick sections. Sections were air-dried on gelatin-coated slides, immersed in hematoxylin for 5 min and checked for complete staining in tap water. Eosin staining was performed for 3 min. Sections were dehydrated through a graded series of alcohols (70–100% ethanol, 3 min each), cleared in xylene and mounted on coverslips. The stained sections were photographed using a Nikon microscope. Five sections from each sample were evaluated.

2.4. Flow cytometry

For the cell surface P-selectin binding assay, neutrophils were washed twice with PBS, then incubated with 2 μg/ml recombinant human P-selectin/Fc chimera protein (rhP-Fc) or human IgG at 4 °C for 30 min. After washing, the cells were suspended in 100 μl PBS containing FITC-labeled goat anti-human IgG (2 μg/ml) and incubated at 4 °C for 30 min. Cells were washed twice and 10 000 cells were counted by flow cytometric analysis with a Beckman FACScan. For inhibition assay of P-selectin binding, rhP-Fc was pre-incubated with mAb 9E1 (positive control) or AC 1.2 (negative control), or various concentrations of BCPs at 37 °C for 30 min.

2.5. Static adhesion assay

CHO-P or CHO cells were seeded in 24-well plates overnight to form monolayers. For inhibition experiments, CHO-P monolayers were pre-incubated with mAb 9E1 or AC 1.2, or various concentrations of BCPs at 37 °C for 30 min. Neutrophils were fluorescently labeled with 5 μM Calcein-AM for 30 min at 37 °C in IMDM. The fluorescently labeled neutrophils were added to CHO-P or CHO monolayers at room temperature for 1 h. After gently washing with PBS, fluorescence was measured by a Molecule Devicer CytoFluor II plate reader using 485 nm excitation and 530 nm emission filters.

2.6. Parallel-plate flow chamber assay

Dynamic interaction between neutrophils and CHO-P cell monolayers were analyzed in a parallel plate flow chamber as described (Tong et al., 2011). CHO and CHO-P cells were seeded in 35 mm culture dishes, respectively, and incubated overnight to form cell monolayers. Blocking mAb (9E1), non-blocking mAb (AC1.2) and BCPs were added onto the cell monolayers, respectively, then incubated at 37 °C for 30 min. The culture dishes were assembled in a parallel-plate flow chamber (GlycoTech, Rochville, MD, USA) and mounted onto an inverted microscope. After washing with PBS, 2×10^6 cells/ml neutrophils were perfused through the flow chamber at the shear stress of 1.2 dyn/cm^2 driven by a syringe pump. The observation fields were randomly selected and recorded for 3 min *via* a CCD-camera (Panasonic, Yokohama, Japan). The number of neutrophils rolling on CHO or CHO-P cells and the relative rolling speed were calculated by NIH ImageJ software from three independent experiments.