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Characterization of a polysaccharide from *Rosa davurica* and inhibitory activity against neutrophil migration

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ABSTRACT

The rapid recruitment of neutrophils from peripheral blood into infected sites is critical step for inflammatory responses; however, the excessive and improper recruitment can lead to serious tissue damages. Thus, it is a promising strategy to inhibit their excessive recruitment for treating inflammation-related disease. Here, we isolated a polysaccharide (RDPA1) from *Rosa davurica*, to evaluate its physicochemical property and inhibitory effects on neutrophil migration. RDPA1 was obtained by hot-water extraction, ethanol precipitation, and fractionated by DEAE-cellulose and Sepharose CL-6B columns. RDPA1 significantly inhibited *in vitro* migration of human neutrophils evaluated by transwell chamber and impacted the migratory behavior observed by time-lapsed microscopy, we found the migrated distance and average velocity of RDPA1-treated cells were greatly reduced. In addition, RDPA1 treatment impaired *in vivo* neutrophil infiltration in the peritonitis mice. RDPA1 exhibited significant blocking capacity of the interaction between β2 integrins and ICAM-1 evaluated by flow cytometry and *in vitro* protein binding assay. Together, these results suggest RDPA1 could be considered as a potential candidate for developing a novel anti-inflammatory agent.

1. Introduction

When infection occurs, neutrophils are rapidly recruited into infection sites, where they destroy the invading microorganisms within a few hours by phagocytosis of pathogens and releasing several antimicrobial chemicals [1,2]. However, the excessive and improper recruitment and activation of neutrophils usually contribute to organ dysfunction, serious tissue injury and inflammation-related diseases, and a poorly controlled acute inflammatory response, such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), even leads to death [3,4]. NSAIDs (nonsteroidal anti-inflammatory drugs) have been widely used for treating inflammation diseases, however, the adverse effects caused by NSAIDs have become increasingly common, such as indigestion, stomach upset (including nausea or feeling sick), stomach pain, gastrointestinal bleeding or ulcers [5]. Therefore, isolation and screening natural compounds from medical herbs with low toxicity and side-effects, which can restrict the excessive and improper recruitment of neutrophil into inflammatory tissue, have been considered as an effective strategy in amelioration of inflammation-related diseases [6].

Natural polysaccharides have been proved to be promising candidates with anti-inflammatory activity, which affect multiple targets during inflammatory progression. For example, polysaccharides from strawberry and mulberry fruit decreased the expression of pro-inflammatory cytokines, such as IL-1β, IL-6 and INF-γ, whereas the anti-inflammatory cytokine, such as IL-10 and MIP-1β, was markedly increased, suggesting that the anti-inflammatory potential of polysaccharides might be *via* modulating pro-/anti-inflammatory cytokine secretion profiles [7]. Sulfated polysaccharide from *Ecklonia cava* inhibited NO production, prostaglandin-E2 (PGE2) production and suppressed inducible iNOS and cyclooxygenase-2 (COX-2) expression [8]. Polysaccharides from *Padina tetrastromatica* reduced the activity of lipoxygenases (LOX) and COX, which are inflammatory marker enzymes, and increased the concentration of serum ceruloplasmin

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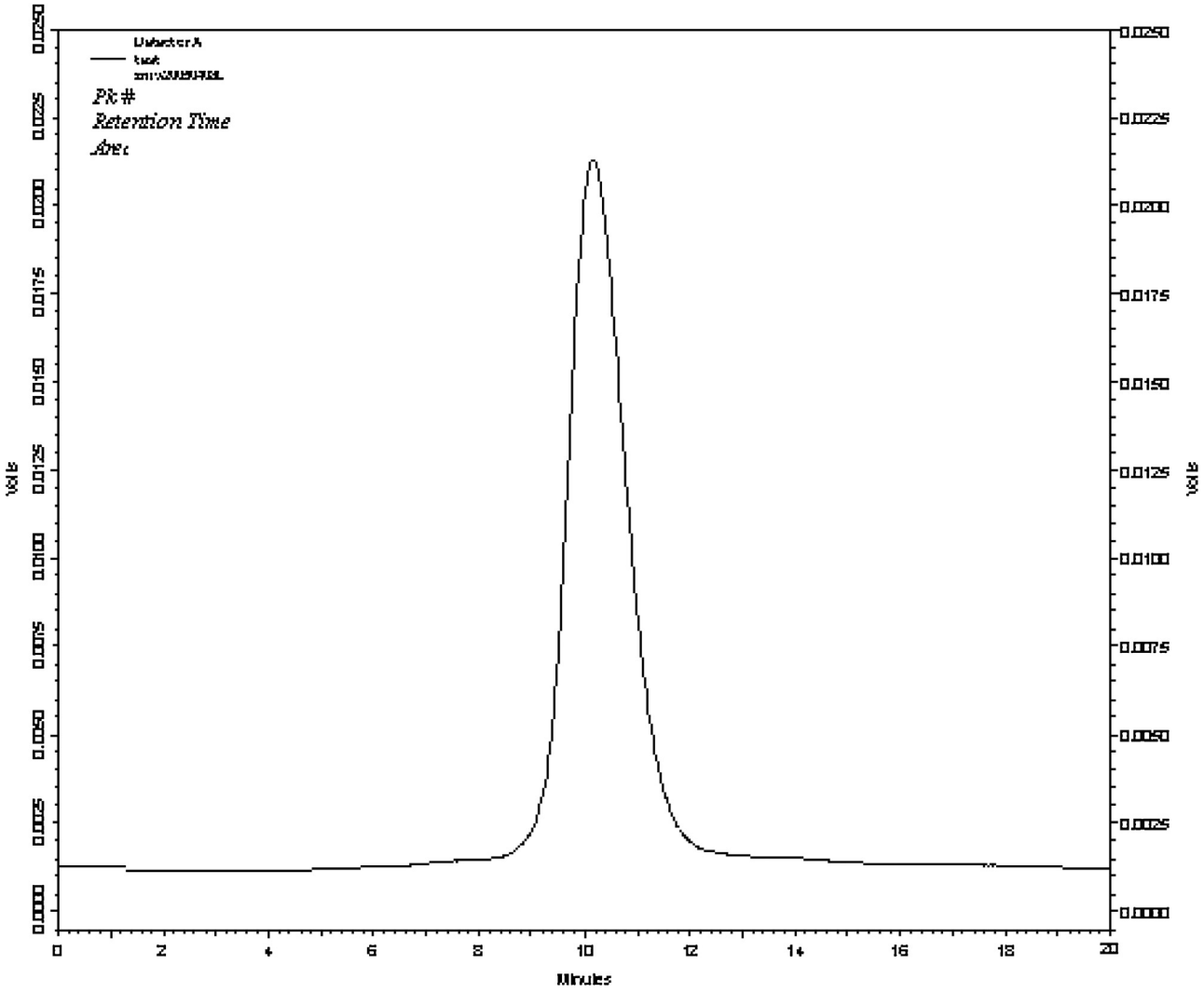

Fig. 1. HPGPC Profile of polysaccharide RDPA1 isolated from *R. davurica* fruits. Homogeneity and molecular weight of RDPA1 was evaluated and determined by HPGPC. Sample was loaded onto Shimadzu HPLC system equipped with a TSK-GEL G3000 PWXL column, eluted with 0.1 M Na_2SO_4 solution and detected by a RID-10A Refractive Index Detector.

and myeloperoxidase (MPO), and also inhibited the nitric oxide synthase (iNOS) and COX-2 expression [9]. These above-mentioned natural polysaccharides showed less toxic feature and significant anti-inflammatory effects. In addition, more and more evidences illustrate natural polysaccharides can interfere with the recruitment and migration of leukocytes into inflammatory sites [10,11].

Rosa davurica is a species of rose native to eastern Asia, in northeastern China, Japan, Korea and southeastern Siberia, where it grows well in a wide range of soil types, including sandy ones, and withstands difficult conditions, including salt and drought. It produces a tart, bright red fruit about the size of a cherry tomato in autumn. The fruit of *R. davurica*, rich in carbohydrates, polyphenol, minerals (magnesium, copper, calcium, iron) and dietary fiber as well as vitamins (thiamin, folic acid, riboflavin, ascorbic acid, niacin), is a famous wild fruit in northeast of China. *R. davurica* has been used as a traditional Chinese herbal remedy for various diseases, and the medicinally beneficial effects of *R. davurica*, such as their antioxidant activities, anti-HIV, and antiviral, antibiotic and hypoglycemic activities, are well known [12–14]. In addition, contemporary pharmacological researches indicate *R. davurica* possesses significant anti-inflammatory effects. However, the active ingredients in *R. davurica* and the mechanisms for anti-inflammation are still unclear. Considering the anti-inflammatory effects of natural polysaccharides through inhibiting leukocyte migration into inflammatory sites, the present experiments were carried out to isolate the polysaccharide from *R. davurica* fruits and further investigate its physicochemical properties and inhibitory effects on neutrophil migration *in vitro* and recruitment *in vivo*.

2. Experimental

2.1. Materials and chemicals

The fruits of *R. davurica* were collected from Jilin City, China. DEAE-Cellulose and Sepharose CL-6B were purchased from Amersham (Sweden). N-formyl-methionyl-leucyl-phenylalanine (fMLP), T-series dextrans, dimethyl sulfoxide (DMSO), bovine serum albumin (BSA) and standard sugars were obtained from Sigma (St. Louis, MO, USA). Calcein acetoxymethylester (Calcein-AM) was purchased from Invitrogen. All other chemical reagents used were analytical grade.

2.2. Isolation and purification of polysaccharide

Freeze-dried *R. davurica* fruits (200 g) were ground and extracted with 80% (v/v) ethanol for 24 h. After filtered, the residues