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Sulfated *Laminaria japonica* polysaccharides inhibit macrophage foam cell formation

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ABSTRACT

In this work, a purified *Laminaria japonica* polysaccharide (LJP61A) was chemically modified to obtain three sulfated polysaccharides (SLJP1, SLJP2 and SLJP3) with different degrees of sulfation using the method of chlorosulfonic acid/pyridine. The effects and underlying mechanism of SLJP1, SLJP2 and SLJP3 on the suppression of macrophage foam cell formation were further investigated using the model of oxidized low-density lipoprotein (ox-LDL)-induced foam cell formation. Results exhibited that the macrophage foam cell formation induced by ox-LDL could be significantly alleviated by these sulfated polysaccharides in a dose-dependent manner. Meanwhile, the enhancement of PPAR-γ mRNA expression in ox-LDL induced macrophages was remarkably inhibited by these sulfated polysaccharides. Moreover, the cellular inflammation induced by ox-LDL could also be remarkably mitigated by these sulfated polysaccharides. These results indicated that the sulfated *L. japonica* polysaccharides could inhibit the conversion of macrophage into foam cell via obstructing PPAR-γ activation and alleviating cellular inflammation.

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1. Introduction

Polysaccharides are one of the most important biomacromolecules, consisting of many monosaccharides (usually more than 10) joined by glycosidic bonds, and widely distribute in plants, animals and microorganisms. In recent years, polysaccharides have attracted a large attention due to their complex structure and various biological activities such as immunoregulatory [1–3], anti-oxidative [4–6], anti-cancer [7], renoprotective [8], and hepatoprotective [9, 10] effects. It is recognized that the biological activities of polysaccharides could be affected by their structural characteristics, such as monosaccharide composition, glycosidic bond linkage, average molecular mass [11]. Therefore, structure modification of polysaccharides to improve their biological activities give rise to wide concern, which resulted in the appearance of several methods, such as sulfation, phosphorylation, acetylation hydroxypropylation and carboxymethylation for synthesizing functionalized polysaccharides [12]. Among them, sulfation was considered as one of the most important methods to synthesize polysaccharide derivatives for further applications because of the sulfated polysaccharides exhibiting diverse remarkable biological activities [13–16].

Laminaria japonica, a famous edible-medicinal marine vegetable in worldwide, has long been recognized as a valuable therapeutic agent for phlegm elimination, detumescence and weight loss [17]. Our previous study indicated that the crude *L. japonica* polysaccharides (LJP) have the ability of anti-atherosclerosis in atherosclerotic mice [17]. Furthermore, it was also observed that a purified *L. japonica* polysaccharide (LJP61A) with known structure exhibited significant inhibitory effect on the conversion of macrophages into foam cells via regulating cellular lipid metabolism and suppressing cellular inflammation [18]. However, the sulfated modification of LJP61A and the biological activities of its sulfated derivatives have not been reported. In the present work, the sulfated LJP61A (SLJP1, SLJP2 and SLJP3) were prepared by chlorosulfonic acid-pyridine (CSA-Pyr) method and their inhibitory effect on the conversion of macrophages into foam cells were further investigated.

2. Materials and methods

2.1. Materials and chemicals

LJP61A was extracted and purified as described previously [18]. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (MO,USA). Fetal bovine serum (FBS), Dulbecco's modified

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Eagle's medium (DMEM), streptomycin and penicillin were obtained from Hyclone Co. (UT, USA). The ox-LDL was obtained from Gaochuang Chemical Technology (Shanghai, China). Trizol Reagent and SYBR Green I detection reagents were purchased from Bio-Rad Co. (CA, USA). All primers and BSA was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All the primary antibodies were supplied by the Cell Signaling Technology Inc. (MA, USA). The secondary antibody was purchased from Boster Co. (Wuhan, China). All other reagents were of analytical reagent and acquired from local chemical suppliers.

2.2. Preparation of sulfated polysaccharides

The sulfuric acid groups of LJP61A were completely removed by the method of Shao et al. [19] to obtain desulfated LJP61A (DS-LJP), which was further used to prepare sulfated LJP61A with various degrees of sulfation according to the previously reported method [20]. Briefly, CAS was slowly added into anhydrous pyridine with continuous stirring and cooling in ice-water bath to obtain the sulfation reagent. DS-LJP (100 mg) was added to 6 mL anhydrous formamide at room temperature with stirring for 30 min. Then, the sulfation reagent (7 mL) was added dropwise and the mixture was stirred for 1, 2 or 3 h at 60 °C. After reaction, the final product was cooled down to room temperature, neutralized with 1 mol/L NaOH solution, and treated by adding 95% EtOH for precipitation. The precipitate was redissolved in deionized water and dialysed with deionized water for 72 h to remove pyridine. The retained non-dialysate was concentrated with the decompression under 60 °C. Then the concentrate was freeze-dried, dialysed and purified to give the final sulfated polysaccharides (named SLJP1, SLJP2 and SLJP3). The sulfur content (S%) was determined by barium chloride-gelatin method [21]. The degree of substitution (DS) was calculated according to the equation: $DS = 1.62 \times S\%/(32–1.02 \times S\%)$. The DS of SLJP1, SLJP2 and SLJP3 were determined as 1.06, 1.28 and 1.56, respectively. And their molecular weights were determined as 1.05×10^4, 1.87×10^4 and 2.36×10^4 kDa.

2.3. Cell line and cell culture

RAW 264.7 macrophages were obtained from Professor Jian Liu (Hefei University of Technology, Hefei, China). The cells were maintained in DMEM containing 10% heat-inactivated FBS, 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) and cultured at 37 °C under the humidified atmosphere of 5% CO_2.

2.4. Evaluation of cell viability

The effects of sulfated polysaccharides on the viability of RAW 264.7 cells were measured by MTT assay [18]. RAW 264.7 cells were seeded in 96-well plates at a density of 5×10^4 cells/mL and cultured with sulfated polysaccharides at the final concentrations of 5, 10, 15, 25, 50, 100 and 200 μg/mL for 24 h. After incubated, 20 μL of MTT and PBS solution (5 mg/mL) was added into each well. After 4 h, 100 μL DMSO was added into all wells. The absorbance was measured at 570 nm on the Bio-Rad model 680 Microplate Reader.

2.5. Oil Red O staining

After treated with ox-LDL (50 μg/mL) in the presence or absence of sulfated polysaccharides (5 and 25 μg/mL) for 48 h, the cells were washed with PBS, fixed with paraformaldehyde (4% in PBS), and stained with Oil Red O.

2.6. Quantitative RT-PCR analysis

According to the reference [22], the quantitative real-time PCR was performed to analyze the mRNA levels of peroxisome proliferator-activated receptor-γ (PPAR-γ), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10) in cells. The mRNA levels were calculated relative to GAPDH mRNA as the invariant control. All primer sequences were listed as follows: PPAR-γ, 5′-TGGCATCTCTGTGTCAACCATG-3′ (upper primer) and 5′-GCATGGTGCCTTCGCTGA-3′ (lower primer);

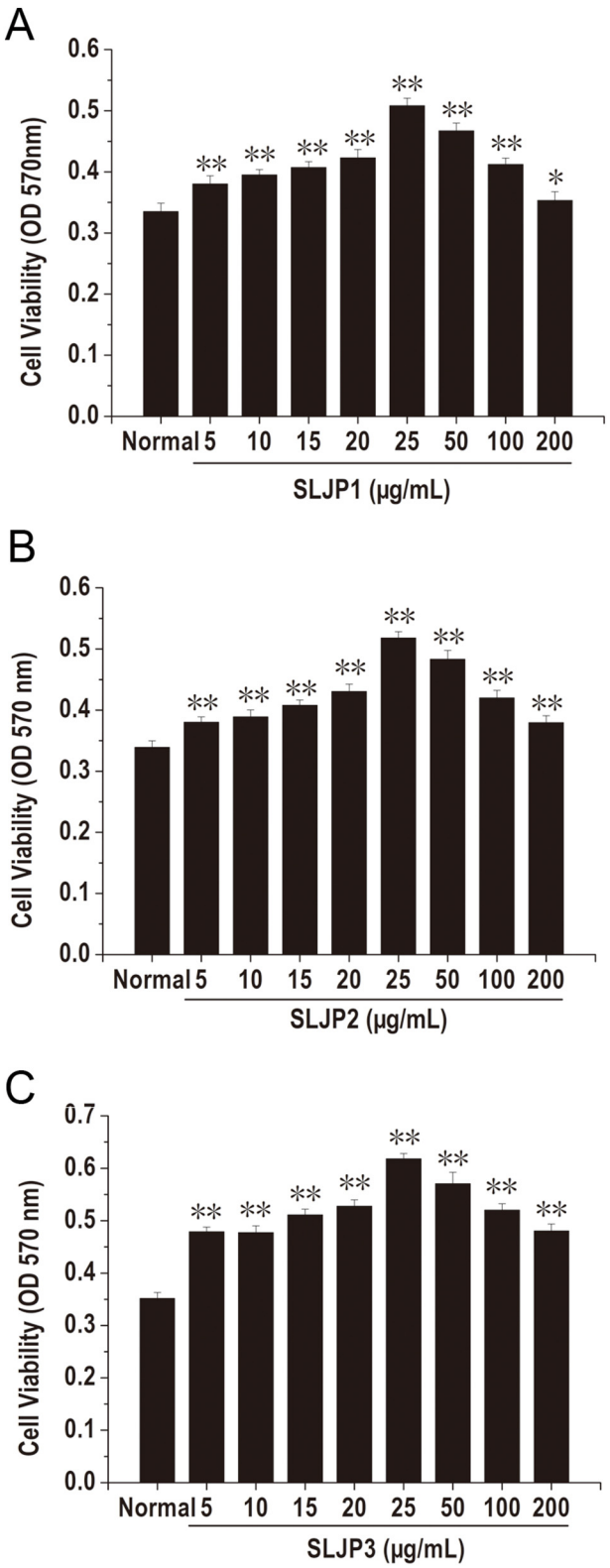

Fig. 1. The effects of sulfated polysaccharides on the viability of RAW 264.7 macrophage cells. Data were expressed as mean ± SD from three independent experiments. $^{**}p < 0.01$ versus normal.

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