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Low molecular weight fucoidan modulates P-selectin and alleviates diabetic nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is a serious microvascular complication that can lead to chronic and end-stage renal failure. It is understood that inflammation is associated with the onset and process of DN. Low molecular weight fucoidan (LMWF) isolated from *Saccharina japonica* has anti-inflammatory properties. Therefore, this study aimed to explore the mechanism of LMWF in DN model induced by streptozotocin. The biochemical indices levels showed LMWF reduced the DN diagnostic indices to protect renal function. The HE stained sections exhibited LMWF protected normal morphological structures and reduced inflammatory cell infiltration in the kidneys of DN rats. Furthermore, the levels of P-selectin and selectin-dependent inflammatory cytokines resulting from LMWF were obviously decreased at both the transcriptional and protein levels. Thus, our results found that LMWF protected the renal function in DN rats and alleviated inflammation through the modulation of P-selectin and inflammatory cytokines. LMWF may have therapeutic potential against DN.

1. Introduction

Diabetic Nephropathy (DN) has increased rapidly in the past decade, with 25–40% of diabetic patients developing the condition and almost 50% of cases with end-stage renal diseases attributable to DN [1–3]. DN is a serious and major microvascular complication caused by diabetes, and it can lead to chronic renal failure and end-stage renal failure [4,5]. Factors that appear to affect DN pathogenesis include proteinuria, inflammation, ischemia, genetics and race [6,7] and those factors contribute to metabolic disorders and kidney cell dysfunction that leads to renal failure [8]. Diabetes induces renal cells to produce various cytokines and numerous inflammatory factors, including transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and intercellular adhesion molecule-1 (ICAM-1) [2,3,6,9,10]. Inflammatory factors influence DN by altering renal cell structure and function in the extracellular matrix (ECM) and the glomerular basement membrane (GBM).

A marine bioactive compound that may offer new therapeutic methodology for DN is fucoidan, a family of sulfated polysaccharides commonly found in brown algae and in several marine invertebrates [11,12] that has attracted interest due to its bioactivities and modern uses [13–15]. One source of fucoidan is the brown algae *Saccharina japonica*, a common seafood in Asia that can control the edema in kidney diseases [16,17].

Fucoidan obtained from *Saccharina japonica* is a heteropolysaccharide composed of an α-L-fucose enriched backbone that mostly contains galactose and sulfates. The fucoidan contains a small quantity of mannoses, glucuronic acid, glucose, rhamnose, arabinose and xylose [18]. Low molecular weight fucoidan (LMWF) is a highly sulfated fraction degraded from fucoidan in *Saccharina japonica* [19]. Sulfation and molecular weight affect the biological activities of polysaccharides [20]. Thus, LMWF has biological activities such as anti-inflammatory [21], antiangiogenic [22,23],

Abbreviation: DM, diabetes mellitus; DN, diabetic nephropathy; ECM, extracellular matrix; GBM, glomerular basement membrane; HE, hematoxylin and eosin; HRP, horse radish peroxidase; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; IHC, immunohistochemistry; JNK, c-Jun amino terminal kinase; LMWF, low molecular weight fucoidan; M-TP, micro total protein; MAPK, mitogen-activated protein kinase; RT-qPCR, real time quantitative polymerase chain reaction; STZ, streptozotocin; TBS, Tris-HCl buffer solution; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; uAlb, urinary Albumin; WB, western blot.

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Table 1
Primary antibodies used for Immunohistochemistry (IHC) and western blot (WB).

Antibody	Source	Company	Dilution (IHC/WB)
β-actin	Rabbit	Proteintech	1: 100/1: 2000
P-Selectin	Mouse	Proteintech	1: 100/1: 2000
IL-6	Rabbit	Proteintech	1: 100/1: 2000
TGF-β	Rabbit	ThermoFisher- Pierce	1: 100/1: 2000
ICAM-1	Rabbit	Proteintech	1: 100/1: 2000
TNF-α	Rabbit	ThermoFisher- Pierce	1: 100/1: 2000
JNK	Rabbit	Proteintech	1: 100/1: 2000

JNK: c-Jun amino terminal kinase.

antithrombus [24,25] and antioxidant [26,27]. LMWF can also modulate cell adhesion [21] factor and growth factor [28].

Although DN has not been traditionally considered to be an inflammatory disease, inflammation may be key in DN. In establishing an inflammatory cycle in the kidney and promoting DN development, selectin-dependent leukocytes roll on the endothelium to initiate leukocyte recruitment, integrin activation, leukocyte adhesion and leukocytes crossing the endothelium [9,29]. Stored in secreted granules of platelets and endothelial cells, P-selectin is a 140 kDa glycoprotein that affects leukocyte recruitment, leukocyte rolling and platelet adhesion [30]. P-selectin has an upstream effect on diabetic retinopathy by increasing glomerulus and interstitial capillaries before activating inflammatory mediators in diabetic human kidneys [31,32]. A natural ligand of P-selectin, fucoidan lowers P-selectin expression by increasing concentrations and decreasing molecular weight of fucoidan [33,34]. LMWF blocks P-selectin function, inhibits leukocyte rolling and accumulation in pneumococcal meningitis and mesentery in rabbits [35,36]. LMWF also activates platelets and promotes revascularization in human cardiovascular diseases [21,37].

In vivo and *in vitro* study on streptozotocin (STZ)-induced DN rats shows LMWF's renoprotective effect [4] and hypoglycemic activity [26,38]. The present study aimed to determine if LMWF alleviates DN and protects renal function by modulating P-selectin and selectin-dependent inflammatory mediators.

2. Materials and methods

2.1. Ethics statement

Experiments involving live animals were conducted in accordance with the "Regulations for the Administration of Affairs Concerning Experimental Animals" promulgated by the State Science and Technology Commission of Shandong Province. The present study was approved by the ethics committee of the Institute of Oceanology, Chinese Academy of Sciences.

2.2. Materials

Drugs and reagents used in the present study were Captopril tablets (Harbin Pharmaceutical Group), STZ (Sigma-Aldrich, Shanghai, China). Primary antibodies are summarized in Table 1. Detection kits: Polink-2 plus polymer horse radish peroxidase (HRP) detection system kit, DAB protein assay kit (GBI, USA), SABC-FITC (POD) Kit (Boster Co., Ltd., China), Rat urinary Albumin (uAlb), IL-6, MAPK (mitogen-activated protein kinase) and TNF-α ELISA kit (Bo Maide Co., Ltd., China). SYBR *Premix Ex Taq*™ II, PrimeScript™ RT reagent Kit and Universal RNA Extraction Kit (TaKaRa Biotechnology Co., Ltd., China). Radio immuno precipitation assay (RIPA) buffer containing phenylmethanesulfonyl fluoride and BCA protein assay kit (Solarbio Science & Technology Co., Ltd., China). Unless otherwise noted, all drugs were of analytical grade and dissolved in normal saline.

2.3. LMWF obtainment

Specimens of *Saccharina japonica* cultured in Rongcheng, China were harvested and immediately transported to the laboratory where LMWF (MW = 8177 Da) was isolated and LMWF structure was determined [39]. Sulfated content was established by ion chromatography on a Shodex IC SI-52 4E column and eluted with 3.6 mM Na_2CO_3 at a flow rate of 0.8 mL/min at 45 °C. Fucose content was determined as described by Wang et al. [39]. Uronic acid (UA) concent was determined by a modified carbazole method. Molecular weight of LMWF (Mw = 8177 Da) was evaluated by GPC—HPLC on a TSK G3000 PWxl column with elution in 0.05 M Na_2SO_4 at a flow rate of 0.5 mL/min at 40 °C with refractive index detection. Ten different molecular weight dextrans purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) served as weight standards. Composition was analyzed as: fucose content 35.07%, sulfate content 36.85%, uronic acid content 0.039%. LMWF was dissolved in distilled water.

2.4. Animal

Adult Wistar male rats (200 ± 20 g body weight, 100 d) from Laboratory Animal Center of Shandong University, China were used in the experiment. All rats were housed in controlled and standard environmental conditions, with room temperature (22 ± 1 °C), relative humidity (60 ± 5%) and a light/dark (12/12 h) cycle. Standard chow diet and water were given *ad libitum*.

2.5. Experimental design

After rats acclimated for one week, the DN model was induced by STZ (50 mg/kg, intraperitoneally, i.p.). Two days later, blood glucose levels from the caudal vein were detected and the detection continued for 3 days. Rats with blood levels > 16.7 mmol/L were considered successful diabetes mellitus (DM) models, randomly divided into five groups and housed in plastic cages (six animals per cage). Group I (Normal group): Normal Wistar rats received identical volume of 0.9% saline; Group II (LF group): DM model rats received 100 mg/kg/d LMWF; Group III (HF group): DM model rats received 200 mg/kg/d LMWF; Group IV (Captopril group): DM model rats received 10 mg/kg/d Captopril, the preferred drug for reducing urine protein and slowing renal function decline; Group V (Model group): DM model rats received identical volume of 0.9% saline. LMWF and Captopril were dissolved in 0.9% saline and orally administered to all groups between 08:00 and 10:00 h for 70 days. Rats were weighed twice a week.

At last, 24-h urine samples were collected from all rats using metabolism cages. Blood samples were drawn from the orbits of all rats before they were anaesthetized with 10% chloral hydrate. Rat kidneys were removed, frozen with liquid nitrogen and fixed in 4% paraformaldehyde for subsequent testing.

2.6. Renal function assessment

2.6.1. Determining urinary micro total protein (M-TP)

24-h urine sample volumes were measured and recorded. Urine was centrifuged and supernatants detected the content of urinary micro total protein using an automatic biochemistry analyzer AU5800 (Beckman Coulter, USA).

2.6.2. Assessing UAlb and inflammatory cytokines

Partial volumes of 24-h urine samples were centrifuged at 3000 rpm for 20 min at room temperature. Proteins obtained from supernatants or kidneys were transferred into uAlb, P-selectin, TNF-α, IL-6 and MAPK rat ELISA kit plates according to manufacturer protocols and the plates were incubated at 37 °C for 30 min.