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Low molecular weight fucoidan ameliorates streptozotocin-induced hyper-responsiveness of aortic smooth muscles in type 1 diabetes rats ☆

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ABSTRACT

Ethnopharmacological relevance: Low molecular weight fucoidan (LMWF) was prepared from *Laminaria japonica* Areschoug, a popular seafood and medicinal plant consumed in Asia. Chinese have long been using it as a traditional medicine for curing hypertension and edema.
Aim of the study: This study was intent to investigate the possible beneficial effect of LMWF on hyper-responsiveness of aortic smooth muscles instreptozotocin (STZ)-induced type 1 diabetic rats.
Materials and methods: Sprague-Dawley rats were made diabetic by injection of STZ, followed by the administration of LMWF (50 or 100 mg/kg/day) or probucol (100 mg/kg/day) for 12 weeks. Body weight, blood glucose level, basal blood pressure, serum lipid profiles, oxidative stress, prostanoids production, and vasoconstriction response of endothelium-denuded aorta rings to phenylephrine were measured by Real time-PCR, Western blots, ELISA assay, and force myograph, respectively.
Results: LMWF (100 mg/kg/day)-treated group showed robust improvements on STZ-induced body weight-loss, hypertension, and hyperlipidaemia as indicated by decreased serum level of total cholesterol, triglyceride, and low density lipoprotein cholesterol; while probucol, a lipid-modifying drug with antioxidant properties, displayed mild effects. In addition, LMWF appreciably ameliorated STZ-elicited hyper-responsiveness and oxidative stress in aortic smooth muscles as indicated by decreased superoxide level, increased glutathione content and higher superoxide dismutase activity. Furthermore, administration with LMWF dramatically prevented cyclooxygenase-2 stimulation and restored the up-regulation of thromboxane synthase and down-regulation of 6-keto-$PGF_{1\alpha}$ (a stable metabolic product of prostaglandin I_2) in the STZ-administered rats.
Conclusion: This study demonstrates for the first time that LMWF can protect against hyperlipidaemia, hypertension, and hyper-responsiveness of aortic smooth muscles in type 1 diabetic rat via, at least in part, amelioration of oxidative stress and restoration of prostanoids levels in aortic smooth muscles. Therefore, LMWF can be a potential adjuvant treatment against cardiovascular complications in type 1 diabetes.

1. Introduction

Diabetes mellitus presents a rapid growing health problem and it is one of the most common causes of vascular disease worldwide (Letchuman et al., 2010; Shaw et al., 2010). Increasing evidence suggests that endothelial dysfunction is one of the primary causes of vascular complications in diabetes, which include hypertension, atherosclerosis, peripheral arterial disease, and poor angiogenesis (Grundy, 2012). At the same time, in diabetic patients, vascular smooth muscles exhibit hypertrophy and hyper-reactivity (Redondo et al., 2005), thus also play a crucial role in the vascular complications. These alterations have been attributed to the

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increased production of reactive oxygen species (ROS), which results from reduced activity of catalase (CAT) and superoxide dismutase (SOD), reduced total glutathione level, and increased activity of glutathione peroxidase (Shi and Vanhoutte, 2008). Therefore, it has been well recognized that a single therapy addressing hyperglycemia, insulin resistance or hyperlipidaemia is not sufficient to tackle diabetes associated vascular diseases. Alternatively, addition of antioxidants has demonstrated additive benefits on cardiovascular protection in diabetes considering that ROS is profoundly involved in the development of diabetes mellitus, hypertension, and atherosclerosis (Johansen et al., 2005; Li et al., 2014).

Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) are the two main isoforms responsible for the production of prostaglandins and thromboxane A_2 (TXA_2), which are potent vasoactive modulators regulating vascular functions. Of the prostaglandins produced in the vascular wall, prostaglandin I_2 (PGI_2) relaxes whereas prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin H_2 (PGH_2) and TXA_2 contract vascular smooth muscles. In addition to producing prostanoids, COX, a redox-sensitive enzyme, generates ROS (Tang et al., 2007; Tang and Vanhoutte, 2008; Van Dyke et al., 2010). The upregulation of COX-1 is responsible for the release of endothelium-derived contracting prostanoids in the aorta of diabetic rats and spontaneously hypertensive rats (Tang and Vanhoutte, 2008). COX-2 is an inducible form and its induction is associated with inflammation, rheumatoid arthritis, and ischemia (Warner and Mitchell, 2004). It has been suggested that COX-2 inhibition improves endothelial function in patients with diabetes, hypertension, and coronary heart disease (Widlansky et al., 2003; Wong et al., 2010). Moreover, COX-2 appears to be mainly responsible for the dysfunction of the vascular smooth muscles in type 2 diabetes (Guo et al., 2005). The current study focuses on endothelium-denuded aorta rings to examine the change of COXs and their productions in diabetic vascular smooth muscles.

Brown seaweeds are a common traditional diet in Asia (mostly in China, Japan and Korea) for centuries, and may contribute to the low prevalence of metabolic syndrome, such as obesity, diabetes, and hypertension in these countries (Cardoso et al., 2015; Kumar and Brown, 2013). In the past thousand years, Chinese take the brown seaweed *Laminaria japonica* as a traditional medicine for edema and hypertension (Yen, 1996). The average intake of *L. japonica* in Japan ranging from 3.0 to 6.9 g/day between 1950 and 1996 has been proven to decrease blood pressure (Kumar and Brown, 2013). Both healthy Japanese preschoolers and hypertensive Japanese patients with higher seaweed intake had lower blood pressure (Wada et al., 2011). In addition, Lim et al. found *L. japonica* could improve postprandial plasma glucose and lipids profiles in hyperlipidaemic adults (Kim et al., 2008). Fucoidans, a class of L-fucose-enriched sulfated polysaccharides extracted from brown seaweeds, have been characterized by a variety of bioactivities *in vitro* and *in vivo*, such as anti-oxidation, anti-inflammation and anti-aggregation (Cumashi et al., 2007; Richard et al., 2006), which all seem to be beneficial for alleviation of diabetes complications. Recently, it was demonstrated that the dietary supplementation of a commercial fucoidan (Haewon Biotech, Inc., Seoul, Korea) decreases levels of plasmatic triglyceride, total cholesterol and LDL-cholesterol (Kim et al., 2014). However, the mechanism of the protective effect of brown seaweeds still needs further study.

As previously described (Cui et al., 2014), low molecular weight fucoidan (LMWF) with weight-average molecular weight ~6500 (Daltons) and number-average molecular weight ~5300 (Daltons), respectively, was supplied by the Institute of Oceanology, Chinese Academy of Sciences. LMWF was produced from the seaweed *L. japonica* J. E. Areschoug (1851) (*Laminariaceae*), which was cultured at the coast of Rongcheng, China, collected in August 2013, authenticated by Dr. Lanping DING and stored as a voucher specimen (No. 83) in the Jerbarium of the Algal Chemistry Department, Institute of Oceanology, Chinese Academy of Sciences in Qingdao of China. LMWF may also be referred to as *haidai* (Chinese), *konbu* (Japanese), *dashima* (Korean) or *kelp* (English). Our previous studies found that the LMWF takes an advantage over the others, because it protects against diabetic nephropathy (Chen et al., 2013; Wang et al., 2014), and exerts antithrombotic effect without impact on coagulation (Zhu et al., 2010). Moreover, our recent studies suggest that LMWF dose-dependently induced endothelium-dependent vasodilation and nitric oxide (NO) synthesis in the aorta of GK (non-overweight type 2) diabetic rats, thus ameliorated diabetes induced hypertension (Cui et al., 2014). During diabetes, oxidative stress and/or COX takes part not only in the dysfunction of the endothelium but also in that of vascular smooth muscles, therefore the present experiments were designed to study the contribution of oxidative stress and COX to the abnormal responsiveness of aortic smooth muscles, observed in streptozotocin (STZ)-induced rats for they manifest stable pathological features that resemble human type 1 diabetes with hyperglycemia, hypertension, and development of vascular complications (Gur et al., 2014; Van Dyke et al., 2010). This study evaluated the *in vivo* effects of LMWF on blood pressure and lipid profiles, and its *ex vivo* effects on endothelium-independent vessel contraction, and measured the levels of ROS and COX-derived prostanoids in the endothelium-denuded aortas, and simultaneously compared them with the effects of probucol, a lipid-modifying drug with powerful antioxidant and anti-inflammatory properties (Cui et al., 2014). We have found that LMWF profoundly ameliorated STZ-induced hypertension, hyperlipidaemia, and activation of vascular redox-prostanoids signaling in the aortic smooth muscles of type 1 diabetic rat.

2. Materials and methods

2.1. Animal ethical approval

This study was approved by the Capital Medical University Animal Care and Use Committee, and all studies were conducted in accordance with “Guide for the Care and Use of Laboratory Animals” adopted by the Beijing government and “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (publication No. 85–23, revised 1996).

2.2. LMWF preparation

Fresh algae were promptly washed, sun-dried and kept in plastic bags at room temperature for use. LMWF was extracted according to the method of Wang et al. (2010) with minor modifications. Briefly, LMWF was prepared using ascorbate and hydrogen peroxide (30 mM, 1:1). After reaction for 2 h, the solution was dialyzed using 3600 Da Mw cutoff dialysis membranes and precipitated with ethanol. The chemical composition and molecular weight of LMWF isolated from *L. japonica* Areschoug are shown in Table 1.

2.3. Materials

Probucol tablets were purchased from Qi Lu Pharmaceutical Co., Ltd (Shandong, China). L-phenylephrine, glutathione (GSH), hydrogen peroxide (H_2O_2), STZ, H_2DCF-DA and SQ29548 were all purchased from Sigma Aldrich. COX-1 (C-20), and COX-2 (C-20), and β-actin antibodies were purchased from Santa Cruz. Rat thromboxane B_2 (TXB_2), SOD, malondialdehyde (MDA), CAT, and 6-keto-$PGF_{1\alpha}$ ELISA kits were purchased from Cayman Chemical.