Journal of Ethnopharmacology 151 (2014) 601–608

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research Paper

Effect of sulfated polysaccharides from *Laminaria japonica* on vascular endothelial cells in psychological stress rats

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ARTICLE INFO

Article history:
Received 15 April 2013
Received in revised form
25 October 2013
Accepted 10 November 2013
Available online 16 November 2013

Keywords:
Sulfated polysaccharide
Vascular endothelial cells
Psychological stress
Adrenalin
Cortisol (PubChem CID: 5754)
Adrenalin (PubChem CID: 5816)
Nitric oxide (PubChem CID: 145068)
Endothelin-1 (PubChem CID: 16212950)
Formaldehyde (PubChem CID: 712)
Hydrogen peroxide (PubChem CID: 784)
Acetylcholine (PubChem CID: 187)

ABSTRACT

Ethnopharmacological relevance: *Laminaria japonica* is a popular seafood and medicinal plant in China. *Laminaria japonica* is used in traditional Chinese medicine to treat and prevent hypertension and edema.
Materials and methods: The vascular protective activity and mechanism of sulfated polysaccharides were studied in adrenalin-induced vascular endothelial damage in rats after psychological stress (PS). Vehicle (sham and PS groups), sulfated polysaccharide from *Laminaria japonica* (LP; 1 mg/kg and 5 mg/kg) and enoxaparin sodium (1 IU/kg, reference drug) were all administered for 10 days. Behavioral changes were recorded. Plasma levels of adrenalin, cortisol, monoamine oxidase (MAO), semicarbazide-sensitive amine oxidase (SSAO), formaldehyde, H_2O_2, nitric oxide (NO), endothelin-1 (ET-1), 6-keto-prostaglandin F_{1a} (6-keto-PGF_{1a}), and thromboxane B_2 (TXB_2) were measured. Endothelium-dependent relaxation of the thoracic aorta was measured and transmission electron microscopy of aortic vessels was performed.
Results: Adrenalin metabolites in plasma were significantly lower ($P<0.01$) in rats after LP administration compared with those in the PS groups. The normalized ratios of plasma NO/ET-1 and 6-keto-PGF_{1a}/TXB_2 were maintained and endothelium-dependent relaxation of the aorta was greatly enhanced after LP treatment ($P<0.05$). Morphological alterations were observed in vascular endothelial cells (VECs) in PS rats, with a higher number of lysosomes and vague mitochondrial cristae compared with those in the sham group. However, these histopathological changes were markedly alleviated after LP treatment.
Conclusions: This study shows a protective effect of LP on VECs in PS rats. LP can regulate plasma levels of NO, ET-1, and 6-keto-PGF_{1a}, enhance endothelium-dependent relaxation, and alleviate histopathological changes of lysosomes and mitochondria in VECs. The potential mechanism of LP on VECs in PS rats is related to its function of reducing metabolites of adrenalin.

1. Introduction

Marine organisms are sources of numerous new compounds with multiple pharmacological properties (Arif et al., 2004; Blunt et al., 2006). They have various bioactivities depending on their composition, overall structure and physicochemical properties (Baba et al., 1990; Ellouali et al., 1993). In particular, sulfated polysaccharides extracted from seaweed are drawing increasing attention in the medical and food supplement areas because of their biological activities. Sulfated polysaccharides from brown seaweeds are generally called fucoidans because they are rich in fucose. These fucoidans are reported to have blood anticoagulant, antithrombotic, antiviral, anticoagulant, antioxidant, anti-inflammatory, and anti-proliferative agents (Almeida-Lima et al., 2010; Barroso et al., 2008; Costa et al., 2010; Cumashi et al., 2007; Dore et al., 2013). *Laminaria japonica* is a type of brown seaweed and is a popular sea food in China and other countries. Over the past years, *Laminaria japonica* has been used in traditional Chinese medicine to treat hypertension (Wang and Zhang, 1980), edema (Wang et al., 2012a), thrombosis (Zhu et al., 2010), and osteoarthritis (Myers et al., 2010; Suszko and Obminska-Mrukowicz, 2013). Moreover, the oncostatic activity of *Laminaria japonica* against some mouse tumors has been described (Lins et al., 2009).

According to previous studies (Barfield et al., 1972; Breuer et al., 2001), a psychological stress rat model in lonely fed animals induces anxiety and depression. When a rat is invaded by an unfamiliar rat, it is more likely to become angry and aggressive. Psychological stress induces hypothalamic–pituitary–adrenal axis (HPA) and sympathetic-adrenomedullary system (SAS) activity

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http://dx.doi.org/10.1016/j.jep.2013.11.014

accompanied by long-term emotional behavioral changes. Increased plasma cortisol levels are an important factor in the persistent emotional arousal induced by intense psychological stress (Wang et al., 2012b). With increasing social competition, the effect of psychological stress on related diseases cannot be neglected, such as cardiovascular, cerebrovascular, endocrine, and mental diseases. When humans are under chronic stress, permanent changes in their physiological responses can lead to disease through immune responses (Rief et al., 2001) and platelet activation (Kishi and Numano, 1989). Stimulation of adrenalin excretion by psychological stress can increase circulating formaldehyde levels, which may be involved in the initiation of endothelial injury, and, subsequently, angiopathy (Yu et al., 1997).

Vascular endothelial cells (VECs) play an important role in maintaining structural and functional integrity of the vasculature. VECs can synthesize and release many types of active materials, which take part in regulating the function of the cardiovascular system. Disturbance of these endothelium-derived substances is an important feature of endothelial dysfunction, as well as the basic pathogenesis of most cardiovascular diseases. Treatment for these endocrine disturbances in the cardiovascular system can effectively inhibit heart and vessel remodeling, and improve the prognosis and quality of life in hypertension, atherosclerosis, and heart failure (Lesniak et al., 2001).

People undergo psychological stress all the time in modern society. Although this psychological stress is moderate and not harmful in most cases, it would insensibly induce VECs injury under a long term impact. We had found that the antithrombotic effect in sulfated polysaccharide from *Laminaria japonica* (LP) pretreatment animals was associated with endothelial protection. In a previous study, LP also had a protective effect on increasingly exogenous adrenalin-induced VEC damage *in vitro* and *in vivo*. However, the mechanism of this protective effect on endogenous adrenalin-induced VEC damage is still unknown. Therefore, the present study aims to investigate the protective effect of LP on damaged VECs in a psychological stress rat model. LP, as a preventive drug for reducing the occurrence of cardiovascular disease risk, has potential application. Just as Aspirin, which is a common clinical drug, it is routinely used for the prevention of cardiovascular diseases.

2. Material and methods

2.1. Isolation and purification of polysaccharide

Laminaria japonica was harvested in June 2011 from the Beibu Gulf, and then dried and comminuted. The extractive procedure of polysaccharides was performed according to our previously reported method (Xie et al., 2011). Briefly, dry powder (50 g) was mixed with 1000 ml of distilled water with 0.02% (w/w) cellulose enzyme, 0.05% (w/w) papain, and 0.05% (w/w) neutral protein enzyme, and then incubated at 70 °C for 6 h. After incubation, the pH of the mixture was adjusted to 10.0 with 10% NaOH, and placed at room temperature for 12 h. The supernatant (A) and sediment of the mixture were separated by centrifugation at 500*g* for 15 min. The sediment was transferred into 500 ml of 10% HCl and placed at room temperature for 4 h. After centrifugation (500*g*, 15 min), the supernatant (B) was collected. Supernatants A and B were combined and precipitated with 80% ethanol. After centrifugation (1100*g*, 15 min), the precipitate was dissolved in 100 ml of distilled water and dried. Finally, the crude extract was subjected to DEAE cellulose column chromatography (2.6×50 cm^2) and eluted with 0.5 M NaCl. The yield of the crude polysaccharide (LP) was 6.53% of raw material. The sugar content of LP was determined to be 71.0%. The sulfate radical content was 83.28 mg/g. The monosaccharide composition consisted mainly of glucose (5.05%), mannose (25.10%), rhamnose (7.37%), galactose (8.46%), and xylose (54.02%). The molecular weight of polysaccharide was 67 kDa.

Enoxaparin injection, which is a sulfated polysaccharide, was used as a positive control because some studies have reported its protective effect on VECs (Iba et al., 2012; Manduteanu et al., 2002). This medicine was produced by Sanofi-Aventis Co. Ltd. (Hangzhou, China).

2.2. Animals

Male adult Sprague-Dawley rats weighing approximately 250–300 g were procured from a local animal supplier and were housed in a temperature-controlled room. The rats were acclimatized for 1 week initially, and fed with a normal diet and tap water *ad libitum*. All animal treatments were conducted in accordance with the *Guide for Care and Use of Animal Laboratory* of Guangxi Medical University in China and were approved by the local ethics committee.

For induced psychological stress, rats were individually housed in clean cages (60 cm × 30 cm × 20 cm) and kept on a reverse 12:12 h light–dark cycle with the lights going on at midnight and off at noon. Each rat was housed in its cage for 10 days to establish home cage familiarity (Barfield et al., 1972). After 10 days, an additional male rat received in the same shipment was used as an intruder in each cage. These rats were in the same body weight range and were used only as intruder males for aggression, which can induce an emotional reaction (e.g., fear, anger, and attack) in PS rats. All aggressive activities took place between 13:30 and 15:30 h during the dark phase of the activity cycle of PS rats. Each period of aggression lasted 20 min. The PS rats did not suffer any physical injuries in addition to the psychological stress.

Animals were divided into five groups. (1) The sham control group (sham) included rats that were living in groups and were intraperitoneally injected with saline twice per day. (2) The psychological stress group (PS) included rats that were lonely fed, and intraperitoneally injected with saline twice per day. (3) The psychological stress treated with low dose LP group (PS+LP 1 mg) included rats that were lonely fed and intraperitoneally injected with LP (1 mg/kg body weight) twice per day. (4) The psychological stress treated with high dose LP group (PS+LP 5 mg) included rats that were lonely fed and intraperitoneally injected with LP (5 mg/kg body weight) twice per day. (5) The psychological stress treated with enoxaparin group (PS+Eno) included rats that were lonely fed and intraperitoneally injected with enoxaparin sodium (1 IU/kg body weight, reference drug, twice per day). All the injections were administered at 9 a.m. and 5 p.m. for 10 days.

2.3. Assessment of behavioral changes

The open field test plays a major role in coping with stress (Kalueff and Tuohimaa, 2005; Van Erp et al., 1994). The open field, constructed of black painted wood, was a 100×100 cm^2 square, divided into 25 (20×20 cm^2) squares with white lines, and was surrounded by a 50-cm high wall. After aggression, all rats were put at the center of the open field and their emotionally reactive state was determined by recording their behavior in 3 min. Horizontal activity, expressed as the number of squares crossed, was monitored using a video tracking system. Vertical activity, expressed as the number of times of rearing, was registered by the researchers. Exploratory activity was defined as the sum of horizontal and vertical activities.