Thrombosis Research 129 (2012) 771–778

Contents lists available at ScienceDirect

Thrombosis Research

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

Regular Article

A comparative study of antithrombotic and antiplatelet activities of different fucoidans from *Laminaria japonica*

Xue Zhao [a], Shizhu Dong [a], Jingfeng Wang [a], Fang Li [a], Anjin Chen [b], Bafang Li [a,*]

[a] College of Food Science and Technology, Ocean University of China, No.5, Yu Shan Road, 266003 Qingdao, Shandong, P.R. China
[b] Qingdao Municipal hospital, No.1, Jiaozhou Road, 266003 Qingdao, Shandong, P.R. China

ARTICLE INFO

Article history:
Received 28 March 2011
Received in revised form 6 June 2011
Accepted 25 July 2011
Available online 26 August 2011

Keywords:
Fucoidan
Antithrombotic
Coagulation
Antiplatelet
Hemorheology
Bleeding

ABSTRACT

Introduction: Fucoidans extracted from brown algae represent an intriguing group of natural fucose-enriched sulfated polysaccharide, with excellent anticoagulant, antimetastatic, antiangiogenic and anti-inflammatory activities. In the present study, we compared antithrombotic activities of four fucoidan fractions with different molecular weight and sulfated ester content from *Laminaria japonica* in an electrical induced arterial thrombosis and their potential mechanism underlying such activity.

Results and Conclusions: In vivo middle molecular weight (MMW) fucoidan fractions with molecular weight about 28000 and 35000 exhibited better antithrombotic activity in electrical induced arterial thrombosis than low molecular weight (LMW) fucoidan LF1 and LF2 (Mw 7600 and 3900). Inhibition of arterial thrombosis occurred at dose of 0.1-0.25 mg/kg for MMW fucoidans, accompanied with moderate anticoagulant activity and significant decrease of whole blood viscosity and hematocrit. The antithrombotic effects of MMW Fucoidans might be related with promotion of TFPI content and decrease of TXB2 content, without affecting platelet aggregation and 6-keto-PGF1α content *in vivo*. In contrast, LMW fucoidans showed a correlation among anticoagulant, antiplatelet and antithrombotic effects *in vivo*. Antithrombotic action of LF1 and LF2 required high dose of 2.5-10 mg/kg, concomitantly with anticoagulant activity and specific inhibition of platelet aggregation *in vivo*. Their antithrombotic effect might be related to their promotion of TFPI and 6-keto-PGF1α, down regulation of TXB2, without affecting hemorheology. These findings suggested that fucoidan fractions with different molecular weight acted on the antithrombotic action by different mechanism. By comparison, highly sulfated fucoidan LF2 with molecular weight of 3900 seemed to be a more suitable choice of antithrombotic drug for its antithrombotic activity accompanied with specific inhibitory activity on platelet aggregation, low anticoagulant activity and low hemorrhagic risk *in vivo*.

The leading causes of death (30% of total) in the world are diseases that involving in heart and blood vessels and, consequently, thrombosis. Most thromboembolic processes require anticoagulant therapy. This explains the current efforts to develop specific and potent anticoagulant and antithrombotic agents. Fucoidans extracted from brown algae (Phaeophyta), sea cucumber (Echinodermata, Holothuroidea) and sea urchins (Echinodermata, Echinoidea) represent an intriguing group of natural fucose-enriched sulfated polysaccharides with potential applications in medicine. Over the past decade, many papers have demonstrated that fucoidan possess excellent biological properties, including anticoagulant, antithrombotic, antimetastatic, antiangiogenic, antiflammatory and antiviral activities [1–3]. Moreover, fucoidan from brown algae have the advantage of low contamination level of virus and /or prions.

It has been documented that marine brown algae is an abundant source of anticoagulant polysaccharides. They contained a variety of sulfated L-fucans with anticoagulant activity [4]. The proposed mechanism of anticoagulant action of fucoidan was predominantly related to the *in vitro* potentiation of the natural inhibitors of activated factor II (thrombin) and activated factor X. Inhibitors included the serpins (antithrombin and heparin cofactor II) [5,6]. However, most of the structural requirements for the anticoagulant activity of fucoidan have not yet been determined, for the fact that these algal polysaccharides are very heterogeneous and difficult to define whether they have repetitive units. Furthermore, the structure of fucoidan varies with different species of algal and different preparation of the polysaccharides, as it is the case for heparan sulfates in vertebrates. Thus, each new sulfated polysaccharide purified from a marine alga is a new compound with unique structure and, consequently, with potential novel biological activities.

It has been reported that the mechanism of fucoidan on antithrombotic action was quite different from heparin. For the high

Abbreviations: LMWH, Low molecular weight heparin; MMW, Middle molecular weight fucoidanfucoidan; LMW fucoidan, Low molecular weight fucoidan; AA, arachidonic acid; ADP, adenosine diphosphate; APTT, activated partial thromboplastin time; TT, thrombin time; PT, prothrombin time; Thromboxane A2, TXA_2; Prostacyclin, PGI_2; TXB2, thromboxane B2; 6-keto-PGF1α, 6-keto prostaglandin F1α; TFPI, tissue factor pathway inhibitor.

* Corresponding author. Tel./fax: +86 532 82031936.
E-mail address: chenlis12@hotmail.com (B. Li).

0049-3848/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2011.07.041

hemorrhagic risk, poor solubility and absorption, high molecular weight fucoidan was degraded by acid hydrolysis and radical process degradation to get middle or low molecular weight fucoidan fractions with well defined structure and good solubility. Mauray reported a fucoidan with molecular weight of 20000 from *Ascophyllum nodosum* showed longer venous antithrombotic property, with lower anticoagulant activity than heparin [7]. LMW fucoidan, especially fractions with molecular weight about 8 kDa obtained by free radical degradation from *Ascophyllum nodosum*, have been reported exerted antithrombotic effects on venous and arterial thrombosis, with significantly low hemorrhagic risk [8–10]. However, the possible mechanism on antithrombotic effect *in vivo* of fucoidan from brown algae still remains unclear. Barroso reported that a non-anticoagulant heterofucan has antithrombotic activity in *vivo*[11]. Zhu demonstrated a LMW fucoidan from *Laminaria japonica* showed a specific inhibition on thrombin induced platelet aggregation in *vitro*[12]. Durand reported a LMW fucoidan by radical process depolymerization from *Ascophyllum nodosum* showed a specific inhibition on tissue factor expression and promotion of tissue factor pathway inhibitor (TFPI) in an arterial thrombosis [10]. Richa also found that a non anticoagulant sulfated galactofucan from the brown alga *Spatoglossum schroederi* showed potent antithrombotic activity, for its potent effect stimulating the synthesis of a highly sulfated heparan sulfate by the endothelial cells of the vascular wall in *vitro*[13]. These findings suggested that research for these polysaccharides cannot be restricted to common coagulation assays used in clinic such as APTT, inhibition of plasma proteases activities, or even one experimental thrombosis model. Each type of sulfated polysaccharide demands a detailed and extensive study of on the variations of effects that could affect the haemostatic system.

Laminaria japonica is a kind of brown algal widely cultured in China and there is nearly 3.6% fucoidan in body. In our previous study, we have prepared fucoidan with various molecular weight and sulfated ester content from *Laminaria japonica* and revealed their antioxidant, hepatoprotective and anti-angiogenic activities [14–16]. Zhu reported a fucoidan fraction from *Laminaria japonica* with molecular weight of 7 kDa obtained by free radical degradation exerted antithrombotic activity in an arterial-venous shunt model [12]. However, the relationship between chemical character and antithrombotic activity in *vivo* of fucoidan from *Laminaria japonica* and possible mechanism are still unclear.

In this present study, two middle molecular weight and two low molecular weight fucoidan fractions with different sulfate ester content were used to assess the effects on arterial thrombosis formation, platelet aggregation and blood circulation and to provide pharmacological evidence for clinical application as antithrombotic agents. In addition, potential underlying mechanism was also investigated to find that fucoidan with different molecular weight acted on the antithrombotic action by different mechanism.

1. Materials and methods

1.1. Reagents and Animals

MMW fucoidan MFa and MFb were produced in our lab and analyzed as follows: fucose (wt/wt) 31.2% and 35.2%; uronic acid content 10.5% and 7.8%; sulphated ester content 28.5% and 34.6%. Their weight-average molecular weight and number-average molecular weight are 28000 and 19000 for MFa and 35000 and 28000 for MFb. LMW fucoidan LF1 and LF2 obtained by radical process degradation in our lab were analyzed as follows: fucose content 42.0% and 30.5% (wt/wt); galactose content 19.8% and 23.9%; uronic acid content 5.3% and 3.7%; sulphated ester content 30.7% and 32.5%.Their weight-average molecular weight and number-average molecular weight are 7600 and 7300 for LF1 and 3900 and 3700 for LF2. Low molecular weight heparin (Fluxum) was product of Alfa Wassermann S.P.A. (Italy). Adenosine diphosphate (ADP) and arachidonic acid (AA) were products from Helena Laboratories (USA). Heparin and bovine thrombin were Sigma products. Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), Prothrombin Time (PT), and fibrinogen kits were Siemns Healthcare Diagnostics products (German). Rat Tissue Factor Pathway Inhibitor (TFPI), Thromboxane B2 (TXB2) and 6-keto-prostaglandin F1α (6-keto-PGF1α) radioimmunoassay kits were products of Beijing Sino-uk Institute of Biological Technology (China). The other chemicals were of analytical grade.

Male Wistar rats weighting 280-300 g were provided by the Center of Medical Laboratory Animal, Shandong University, China. Animals were acclimated for at least 1 week at a temperature of 24 ± 1 °C and humidity of 55 ± 5%. The animals were maintained with free access to standard diet and tap water. The study protocol was approved by the Ethics Committee of the Ocean University of China (Shandong, China).

1.2. Treatments

Rats received an intravenous administration of heparin (1, 2 mg/kg), LMWH (25, 50 IU anti-Xa/kg), MMW fucoidan (0.1, 0.25 0.5 mg/kg) and LMW fucoidan (2.5, 5, 10, 15 mg/kg). The control rats were given saline. All experiments *in vivo* were conducted 5 min after the treatment.

1.3. In vivo electrical induced arterial thrombosis

Male Wistar rats were anesthetized with intraperitoneal injection of 20% ethyl p-aminobenzoate. Through a medline cervical incision, the right carotid arteries of rats were exposed via blunt dissection, and carefully dissected clear of the vagus nerve and surrounding tissue. Heparin, LMWH and fucoidan solutions were injected intravenously in femoral vein. Saline was injected intravenously for control rats. A total of 5 minutes after the injection, carotid artery was put into the groove of flow probe and touched with the electrode in the bottom of the probe (Yiyan Technology Company, China). The volume blood flow and heart rate (beats/min) was measured by the infrared detector in the probe. Then a 1.0 mA constant current was delivered for 10 min via the electrode attached to an Animal Thrombosis Generator (YLB, Yiyan Technology Company, China). A calculated occlusive rate (%) of carotid blood was recorded very 4 seconds by infrared detector and mean times for formation of an occlusive thrombus (occlusive rate 95%) in the carotid artery were recorded.

1.4. Determination of plasma TFPI, TXB2 and 6-keto-PGF1α levels

A total of 5 min after male Wistar rats received intravenous administration of saline, heparin, Fluxum and fucoidans for control rats and treated rats, the arterial thrombosis was induced by electrical stimulation for 10 min. The normal group only received a intravenous administration of saline. Next, blood samples of normal, control and treated rats were collected from femoral artery into 3.8% sodium citrate and the serum was obtained by $664 \times g$ for 10 min. The plasma TFPI level was measured by use of radioimmunoassay kit and TFPI level was expressed as ug/mL. Plasma thromboxane A2 (TXA_2) and prostacyclin (PGI_2) were estimated by measuring their stable hydrolysis products TXB2 and 6-keto-PGF1α by radioimmunoassay kits. TXB2 and 6-keto-PGF1α levels were expressed as pg/mL.

1.5. In vitro human platelet aggregation

Human blood was collected from healthy adult volunteers who had not taken any drugs for at least 15 days and the investigation was performed according to the guidance of for the Use of Human Blood published by Shandong Municipal Government. Human blood was added into 3.8% sodium citrate and the platelet-rich plasma (PRP) was obtained by centrifugation ($47 \times g$) for 10 min. The remaining blood was further centrifuged at $664 \times g$ for 10 min to prepare platelet-poor