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Low Molecular Weight Fucoidan Prevents Neointimal Hyperplasia in Rabbit Iliac Artery In-Stent Restenosis Model

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***Objective*—**Smooth muscle cell (SMC) proliferation within the intima is regulated by heparan sulfates. We studied a low molecular weight (LMW) fucoidan (sulfated polysaccharide from brown seaweed) on SMC proliferation in vitro and intimal hyperplasia in vivo.

***Methods and Results*—**In vitro study revealed that LMW fucoidan reduces rabbit SMC proliferation and is internalized in SMC perinuclear vesicles. On rabbit iliac arteries perfused in vivo with fluorolabeled LMW fucoidan after angioplasty, the labeling was mainly located on sites of injury. Pharmacokinetic studies showed that LMW fucoidan exhibited in rats an elimination half-life of 56±25 minutes (n=8) after intravenous administration and a constant plasma rate for ≥6 hours after intramuscular administration. After stent implantation in their iliac arteries, rabbits were also treated with LMW fucoidan (5 mg/kg IM twice a day). Histomorphometric analysis at day 14 indicated that LMW fucoidan reduced intimal hyperplasia by 59% (1.79±0.4 versus 0.73±0.2 mm^2, $P<0.0001$) and luminal cross-sectional area narrowing by 58% (0.38±0.08 versus 0.16±0.04, $P<0.0001$). Blood samples showed no anticoagulant activity due to LMW fucoidan.

***Conclusions*—**This natural polysaccharide with high affinity for SMCs and sustained plasma concentration markedly reduced intimal hyperplasia, suggesting its use for the prevention of human in-stent restenosis. **(*Arterioscler Thromb Vasc Biol*. 2002;22:1604-1609.)**

Key Words: fucoidan ■ hyperplasia ■ restenosis ■ stent ■ vascular smooth muscle cell proliferation

Intimal hyperplasia due to migration and proliferation of smooth muscle cells (SMCs) from the media to the intima is a major component of restenosis after stent implantation.[1,2] Polysaccharides constitute a large family of molecules capable of developing molecular interactions with cellular targets within the arterial wall.[3] For instance, heparin, a natural sulfated polysaccharide, displays pleiotropic effects independent of the anticoagulant activity, including SMC growth inhibition,[4] anti-inflammatory activity,[5] and growth factor protection.[6]

Fucoidan is a sulfated polysaccharide extracted from brown seaweed that reduces rat SMC proliferation in vitro in a more intensive manner than heparin.[7] We recently succeeded in producing and characterizing new homogeneous fractions of low molecular weight (LMW) fucoidan with low anticoagulant activity.

The aims of the present study were to investigate the ability of LMW fucoidan to regulate vascular SMCs. For this purpose, we first tested the ability of LMW fucoidan to inhibit rabbit SMC proliferation in vitro. We explored the pharmacokinetics of LMW fucoidan injected in rats and the effect of LMW fucoidan on intimal hyperplasia. The results described below indicate that LMW fucoidan is a strong inhibitor of intimal hyperplasia and may be potentially relevant for the treatment of in-stent restenosis.

Methods

Polysaccharides

LMW fucoidan was isolated and hydrolyzed by a radical depolymerization process[8] from high molecular weight (HMW) extracts of brown marine algae. The characteristics of LMW fucoidan according to previously reported analytical methods[9] are as follows: weight-average molecular mass 8±1 kDa; fucose content 35% (wt/wt); uronic acid content 3% (wt/wt); and sulfate content 34% (wt/wt). The anticoagulant activity in vitro of the LMW fucoidan was measured by an activated partial thromboplastin time (APTT), and the amount of LMW fucoidan required to obtain an APTT of 80 seconds (control 40 seconds) was 25 μg/mL.[10] The anticoagulant activity in vivo of LMW fucoidan was measured in rabbits by APTT and prothrombin time after intramuscular injection of 5 mg/kg LMW fucoidan. LMW fucoidan was fluorolabeled with the use of 5-([4,6-dichlorotriazin-2-yl]amino)fluorescein.[7,11] LMW heparin (6±2 kDa) was commercially available from Sigma Chemical Co.

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Cell Culture

Eagle's minimum essential medium (MEM) and trypsin-EDTA were purchased from GIBCO. Calf serum was obtained from Eurobio. SMCs were isolated from the abdominal aorta of male New Zealand White rabbits.[12] Cells were cultured at 37°C in a humidified atmosphere (5% CO_2) in MEM supplemented with 10% serum and 2 mmol/L L-glutamine. Third-passage cultures were used throughout the present study.

Growth Studies

Rabbit SMCs (1.2×10^4) in MEM containing 10% serum were growth-arrested by placing them in medium with 0.4% serum for 72 hours. Cell growth was followed in MEM+10% serum with or without polysaccharides. Toxicity was evaluated by trypan blue and clonal growth assay as described.[7] Thymidine uptake at 24 hours was assessed in quadruplicate wells with 1 μCi per well of 6-[^{3}H]thymidine that was pulse-labeled for 60 minutes. Cells were washed, DNA was precipitated with 10% trichloroacetic acid, and radioactivity was counted. Inhibition of SMC proliferation was assessed at day 3 by cell counting of serum-stimulated SMCs in the absence or presence of LMW fucoidan or LMW heparin.

Confocal Study

SMCs plated at low density on coverslips were incubated at 37°C with fluorescent LMW fucoidan (5 μg/mL) for the indicated times, washed 4 times with PBS containing 0.2% BSA, and fixed in 3.7% formaldehyde solution.[7] Cultured SMCs were observed under a confocal microscope (Leica SP; excitation wavelength [λex] 488 nm, emission wavelength [λem] 500 to 590 nm).

Pharmacokinetic Experiments

Animal protocols were approved by the Institutional Animal Care and Use Committee of Faculté Bichat. Adult male Wistar rats (280 to 300 g) were anesthetized with intraperitoneal pentobarbital (0.1 mL/kg). A silicon-tipped polyethylene catheter was inserted into the right jugular vein. The bladder was exteriorized and catheterized. A bolus of 5 mg/kg fluorescent LMW fucoidan was injected (1) intravenously via the jugular vein catheter followed by saline solution to flush the catheter or (2) intramuscularly in the right leg. Blood samples were obtained from the catheter at 0, 15, and 30 minutes and at 1, 2, 4, and 6 hours. Urine was collected via the bladder catheter. Blood was centrifuged, and the serum was obtained. The concentration of fluorescent LMW fucoidan was measured in the plasma and urine by a spectrofluorometer at λex and λem of 489 and 515 nm, respectively.

Pharmacokinetic Analysis

Equation 1 describes the time course of fluorescent LMW fucoidan concentration in the blood according to a 2-compartment open model: $Ct=Ae^{-\alpha t}+Be^{-\beta t}$ (1), where A and B are constants. The parameters were determined using the nonlinear least-squares program Micropharm.[13]

The elimination half-time period, $t_{1/2}\beta$ (β phase), corresponds to the time taken for the concentration of drug in plasma to decline to half of its original value and was calculated from the parameter β by Equation 2: $T_{1/2}\beta=\mathrm{Log}2/\beta$ (2).

The clearance (Cl) is the elimination of the drug in milliliters per minute expressed by Equation 3: $Cl=AUC/D$ (3) where AUC is the area under the curve and was calculated by using the trapezoidal rule by the Micropharm program, and D is the administered dose of LMW fucoidan. The apparent distribution volume is the ratio between the amount of drug in a whole organism and its blood or plasma concentration measured at the same time. Percentage of urinary excretion was calculated from the administered dose and the urinary excreted dose.

Angioplasty and Stenting Protocol

Male New Zealand White rabbits (3.5 to 4 kg) received a standard diet. Animals were anesthetized with intravenous pentobarbital, and the right carotid artery was catheterized with a 5F sheath. A 3.0-mm-diameter 20-mm-long angioplasty balloon catheter was advanced over a standard 0.014-in flexible guidewire in both iliac arteries. The iliac arteries were injured by 3 successive 1-minute inflations at 10 atm with a 1-minute reperfusion phase after each inflation. In some experiments, 1 mL fluorescent LMW fucoidan (5 mg/mL) was locally delivered with a 3-mm annular balloon catheter (Nycomed) in the rabbit iliac artery after angioplasty. After 5 minutes, a segment of damaged iliac artery and an adjacent segment of artery were excised, washed with PBS, and observed under a fluorescent microscope. For the stented animals, a 15-mm-long metallic stent (Helistent-Hexacath, Reuil Malmaison) mounted over the balloon was implanted in both iliac arteries immediately after balloon angioplasty (30-second inflation at 10 atm).[14] LMW fucoidan at 5 mg/kg was injected intramuscularly twice a day for 14 days. Five animals (2 stents per animal) were treated with LMW fucoidan for 14 days. Five control animals received saline.

Tissue Harvest and Histology Processing

Fourteen days after stenting, rabbits were killed by pentobarbital overdose. Iliac arteries were perfusion-fixed and impregnated in methacrylate as described.[14] Four-micron arterial sections were cut with tungsten carbide knives and stained with hematoxylin-eosin, Masson's trichrome, or orcein. In each artery, 3 sections were taken: 2 at 2 mm from the distal ends of the stent and 1 in the middle. Cell density in the neointima was observed under a microscope and analyzed (Lucia Software, Nikon) for 10 different sections per group.

Morphometric Analysis

Digital planimetry with the use of a video camera mounted on a microscope analyzed the borders of the external elastic lamina, internal elastic lamina, and vessel lumen. A computer program allowed quantification of the intimal, medial, and luminal areas.[14] Intimal growth was estimated by using the intimal area, the luminal area, the ratio of intimal to medial areas, and the ratio of the intimal area to the area bounded by the internal elastic lamina (luminal cross-sectional area narrowing).

Statistical Analysis

Results are expressed as mean±SD. The significance of the differences between groups was analyzed by 1-way ANOVA followed by post hoc tests. Values of $P<0.05$ were considered significant.

Results

LMW Fucoidan Inhibits Rabbit SMC Growth In Vitro

We tested the ability of LMW fucoidan to inhibit rabbit SMC growth in vitro. No cell toxicity was observed even at the highest dose (1000 μg/mL). [^{3}H]Thymidine uptake indicated that SMC DNA synthesis 24 hours after serum stimulation was significantly ($P<0.001$) inhibited in the presence of LMW fucoidan in a dose-dependent fashion: 28±2%, 52±3%, and 78±2% inhibition at 10, 100, and 1000 μg/mL, respectively. LMW heparin was less potent on the inhibition of DNA synthesis with 21±2%, 31±4%, and 49±3% inhibition at 10, 100, and 1000 μg/mL, respectively. By cell counting at day 3, we found that LMW fucoidan was a better SMC growth inhibitor than LMW heparin (Figure 1).

LMW Fucoidan Is Internalized in Cultured Rabbit SMCs

Because the growth-inhibitory effect of LMW fucoidan could be, at least in part, due to its internalization in SMCs, we first investigated its uptake by SMCs in vitro. The fate of LMW fucoidan added to cultured rabbit SMCs at 37°C was studied by confocal microscopy using 5 μg/mL of fluorescent LMW

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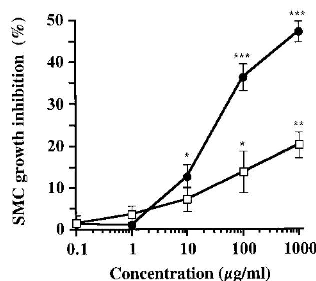

Figure 1. Growth inhibition vs polysaccharide concentration. Rabbit SMCs were released from the growth-arrested phase by addition of culture medium plus 10% serum in the presence of indicated concentrations of LMW fucoidan (●) or LMW heparin (□). Cell number was measured after 3 days of incubation. Growth inhibition was calculated by the ratio of cells in the presence of polysaccharide to cells in the control condition. Values are mean±SD of 3 experiments. *$P<0.05$ vs control, **$P<0.001$ vs control, and ***$P<0.0001$ vs control.

fucoidan. After incubation of SMCs, LMW fucoidan was internalized by endocytosis in fluorescent vesicles clearly seen at 6 hours (Figure 2A). The number of fluorescent vesicles in the perinuclear region increased at 24 hours, but nuclear internalization was never observed (Figure 2B).

LMW Fucoidan Is Found on Injured Segments of Arteries After Balloon Angioplasty

We then investigated whether LMW fucoidan has an affinity for the arterial wall in vivo. A local delivery catheter was used to deliver fluorescent LMW fucoidan in a rabbit iliac artery after angioplasty. By fluorescence microscopy, an intense fluorescence was revealed on the segment damaged by angioplasty, whereas low fluorescence intensity was detected on the adjacent uninjured segment of the artery (data not shown).

A 2-Compartment Pharmacokinetic Model Describes LMW Fucoidan Distribution In Vivo

The serum concentrations of LMW fucoidan after intravenous or intramuscular injection in rats are displayed in Figure 3A and 3B, respectively. After intravenous injection, LMW

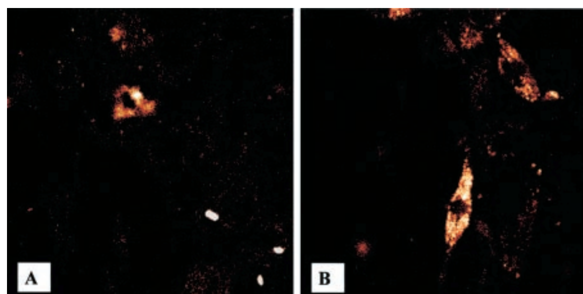

Figure 2. Uptake of fluorolabeled LMW fucoidan in cultured rabbit SMCs. Rabbit SMCs were exposed at 37°C to 5 μg/mL fluorescent LMW fucoidan for 6 hours (A) or 24 hours (B). They were then fixed with formaldehyde and observed by using a fluorescence confocal microscope. Fluorescent endocytotic vesicles were localized in the perinuclear region 6 hours after exposition to fucoidan (A). The number of fluorescent vesicles increased at 24 hours (B), but nuclear internalization was never observed. Original magnification ×1250.

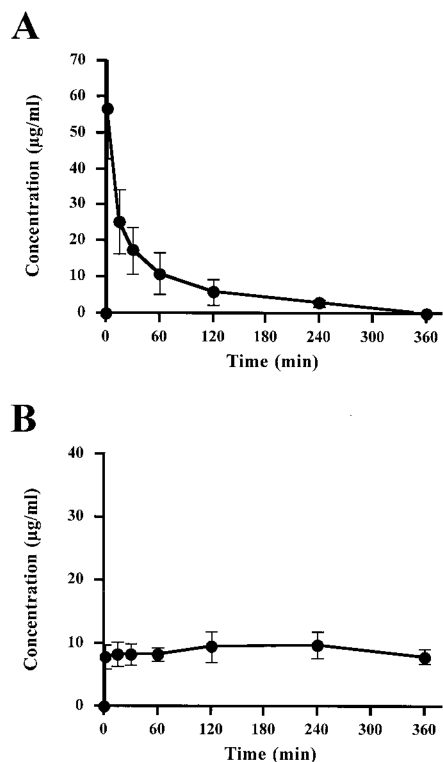

Figure 3. Plasma concentrations of fluorolabeled LMW fucoidan after intravenous (A) or intramuscular (B) injection in rats. Adult male Wistar rats (280 to 300 g) were injected intravenously via the jugular vein catheter or intramuscularly in the right leg with a bolus of 5 mg/kg fluorolabeled LMW fucoidan. Blood samples were collected, and concentration of LMW fucoidan was measured by a spectrofluorometer at λex and λem of 489 and 515 nm, respectively. Data of plasma concentrations are presented as mean±SD (n=8).

fucoidan exhibited a biphasic decline with a rapid decrease of plasma concentration 2 hours after injection and a secondary slow linear decrease (Figure 3A). The pharmacokinetic parameters on 8 adults Wistar rats were as follows: clearance 0.88±0.46 mL/min, $t_{1/2\beta}$ 56.5±25.0 minutes, and distribution volume 57.6±27.9 mL. Urinary excretion of LMW fucoidan was 33.2±2.1%. After intramuscular injection, plasma concentration of LMW fucoidan remained in the 10-μg/mL range for at least 6 hours (Figure 3B). Pharmacokinetic analysis revealed that a 2-compartment model described best the plasma behavior of LMW fucoidan.

LMW Fucoidan Prevents In-Stent Intimal Hyperplasia in Rabbit Iliac Arteries

No rabbit died during the procedures or the 14-day treatment with LMW fucoidan (5 mg/kg IM). Prothrombin time and APTT were not different in blood samples obtained from LMW fucoidan–treated animals versus control animals (data not shown).

In the animal model, angioplasty (3 successive inflations at 10 atm) and stent implantation for 14 days in rabbit iliac arteries increased ($P<0.0001$) the intimal area of these arteries (1.79±0.43 mm^2) compared with arteries in uninjured animals (0.013±0.002 mm^2), whereas the medial area was not different (0.48±0.06 mm^2 in uninjured group versus 0.39±0.09 mm^2 in stented group).

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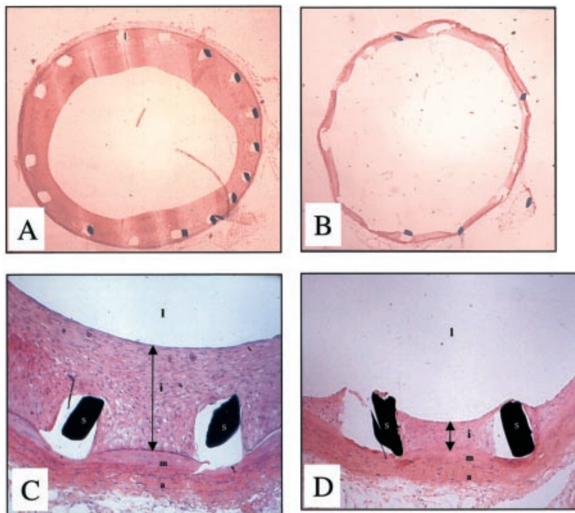

Figure 4. Representative photomicrographs of rabbit iliac arteries 14 days after angioplasty and stenting in control group (A and C) and in fucoidan-treated group (B and D). a indicates adventitia; i, intima, l, lumen; m, media; s, stent; and ↔, intimal thickness. Original magnifications ×12 (A and B) and ×66 (C and D).

After angioplasty and stent implantation, LMW fucoidan treatment after 14 days (Figure 4) was associated with a thinner ($P<0.001$) fibrocellular neointima (101±20 μm) compared with control stenting (236±90 μm). Cell density in the intima was not different ($P=0.5$) between the 2 groups of stented animals, but the significant decrease of the size of the intima by fucoidan treatment suggested an important limitation of the total number of invading and proliferating SMCs. Morphometric analyses indicated that arterial cross-sectional area was similar in the 2 groups, indicating that stent-induced arterial injury was of similar magnitude in the 2 groups without adverse effect of LMW fucoidan on media integrity (Table). Interestingly, intimal area with LMW fucoidan was significantly ($P<0.0001$) reduced by 59±11%, the intima/media ratio was reduced by 56±12%, and luminal cross-sectional area narrowing was reduced by 58±10% (Table). In turn, the luminal area of arteries from LMW fucoidan–treated animals was increased (Table, Figure 4).

Discussion

The results described above indicate that LMW fucoidan is a strong inhibitor of intimal hyperplasia in vivo and may be potentially relevant in the treatment of in-stent restenosis. Fucoidans are a family of L-fucose–containing sulfated polysaccharides extracted from brown seaweed, *Ascophyllum nodosum*.[8] This vegetal polymer shares chemical analogies with heparin, a sulfated polysaccharide of animal origin, but exhibits lower anticoagulant activity.[7]

Morphometric Analysis of Rabbit Stented Iliac Arteries After 14 Days

	Control Stented Arteries	Fucoidan Treatment
Media, mm^2	0.39±0.09	0.37±0.19
Intima, mm^2	1.79±0.43	0.73±0.20†
Intima/media	4.87±1.50	2.14±0.55†
Intima/IEL	0.38±0.08	0.16±0.04†
Luminal area, mm^2	2.97±0.72	3.95±0.65*

IEL indicates internal elastic lamina.
*$P<0.01$, †$P<0.0001$ vs control (n=10 arteries, 30 sections).

Continuous administration of HMW fucoidan (>100 kDa) has been shown to reduce intimal hyperplasia after balloon injury in rats.[15] Nevertheless, the requirement for continuous administration limits the use of HMW fucoidan for pharmaceutical and clinical applications. In addition, restenosis was induced in the study of McCaffrey et al[15] with angioplasty alone, ie, without stent implantation. Surprisingly, there are no published data on the inhibitory effect of LMW fucoidan on intimal hyperplasia. Thus, we decided to test the efficacy of LMW fucoidan on intimal growth after stenting in the rabbit, a model that is more relevant to modern clinical restenosis than is balloon abrasion of the rat carotid artery. The data presented indicated that LMW fucoidan reduced neointimal hyperplasia by ≈60% and increased the luminal area by ≈25%, making this compound one of the most efficient ever tested in experimental restenosis by using a noncontinuous administration regimen.[16] In contrast to heparin tested in animal models of balloon angioplasty[17,18] and clinical trials,[19] HMW fucoidan was already used at high dose (25 mg/kg in mice,[20] 100 mg/kg in rats,[21] and 10 mg/kg per hour in rabbits[22]) without bleeding. In the present study, the fraction of LMW fucoidan (8 kDa), used in rabbits at low dose (5 mg/kg), exhibited no anticoagulant activity in vivo.

In agreement with previous studies using HMW fucoidan in cultured rat and human SMCs,[7,23,24] LMW fucoidan reduced rabbit SMC proliferation in vitro and appeared to be a more antiproliferative agent than LMW heparin. Our confocal microscopy studies show that LMW fucoidan has high affinity for cultured SMCs and is internalized in perinuclear endocytotic vesicles. In a previous study using a 20-kDa fucoidan fraction,[7,25] we demonstrated that the antiproliferative effect of fucoidan on rat SMCs was mediated by its binding to membrane sites, probably similar to those that mediate the endocytosis of heparin.[12,26] Perinuclear localization of LMW fucoidan suggests an antiproliferative activity via intracellular signaling pathways.[23,24] We also observed that after angioplasty and local injection of LMW fucoidan in rabbit iliac arteries, the polysaccharide was located on the injured segment of the artery, whereas a low binding was noticed on the artery without angioplasty. This preferential binding in vivo after injury by angioplasty strongly suggested a favorable location of the LMW fucoidan to inhibit SMC activation and proliferation. McCaffrey et al[27] suggested that antiproliferative activity of HMW fucoidan on rat and bovine aortic cultured SMCs was linked to the protection of transforming growth factor-β1 from proteolytic degradation by plasmin and trypsin. We have previously observed an action of HMW fucoidan on growth factors, such as fibroblast growth factor-1 and fibroblast growth factor-2, leading to an increase in endothelial cell proliferation in vitro[28] as well the release of tissue factor pathway inhibitor from cultured human endothelial cells,[29] which may be relevant in vivo for LMW fucoidan. In vitro and in vivo signaling pathways for

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the inhibition of SMC growth and migration by LMW fucoidan remain to be fully characterized.

The antiproliferative effect on SMCs of LMW fucoidan probably does not fully explain its potent inhibitory effect on intimal hyperplasia. At least 4 other features of fucoidan may play a role as well: (1) The 2-compartment model that best described the plasma behavior of LMW fucoidan after intravenous administration suggests a rapid organ or cellular uptake followed by a slow decrease. Heparin is cleared through a combination of a rapid mechanism (via endothelial cells and macrophage) and much slower first-order mechanisms via renal excretion.[30] Prolonged high plasma rates of LMW fucoidan after intramuscular administration may also facilitate molecular interactions with membrane receptors present on SMCs, which are in contact with blood after the destruction of the endothelial layer by angioplasty. (2) HMW fucoidan is known to modulate leukocyte activation and adhesion via interaction with selectins.[31] Monocyte activation and adhesion are important stimuli in in-stent restenosis.[32] It has been demonstrated that anti-inflammatory cytokines and monoclonal antibody targeted against monocyte integrins inhibit monocyte infiltration and reduce in-stent intimal hyperplasia.[14] HMW fucoidan reduces in vivo neutrophil adhesion and leukocyte migration.[33,34] Our recent data indicate that sulfated polysaccharides, including fucoidan, inhibit the release of proinflammatory cytokines/chemokines by activated monocytes,[35] which may be relevant for the inhibition of intimal hyperplasia.[36] (3) We and other groups have described that HMW and LMW fucoidans have antithrombotic activities and reduce platelet aggregation, an important stimulus of restenosis.[10,29,37] (4) HMW fucoidan increases the mobilization of stem/progenitor cells[38] and increases plasma levels of stroma-derived factor 1, a highly potent chemoattractant for leukocytes and stem/progenitor cells.[39] Because circulating progenitor cells are involved in reendothelialization of injured arteries,[40] a mobilization by LMW fucoidan may result in reduced intimal growth.

In conclusion, we reported in the present study promising results of intimal hyperplasia inhibition with a LMW fucoidan in stented rabbit iliac arteries. The low anticoagulant activity, the favorable safety profile, the potent antiproliferative effect on SMCs, and the potential anti-inflammatory properties of LMW fucoidan make it a promising candidate for prevention of in-stent restenosis either systematically or locally via local delivery catheters or coated stents.[41,42]

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References

1. Casscells W. Migration of smooth muscle and endothelial cells: critical events in restenosis. *Circulation*. 1992;86:723–739.
2. Post MJ, Borst C, Kuntz RE. The relative importance of arterial remodeling compared with intimal hyperplasia in lumen renarrowing after balloon angioplasty: a study in the normal rabbit and the hypercholesterolemic Yucatan micropig. *Circulation*. 1994;89:2816–2821.
3. Blondin C, Bataille I, Letourneur D. Polysaccharides for vascular cell targeting. *Crit Rev Ther Drug Carrier Syst*. 2000;17:327–375.
4. Castellot JJ, Cochran DL, Karnovsky MJ. Effect of heparin on vascular smooth muscle cells, I: cell metabolism. *J Cell Physiol*. 1985;124:21–28.
5. Salek-Ardakani S, Arrand JR, Shaw D, Mackett M. Heparin and heparan sulfate bind interleukin-10 and modulate its activity. *Blood*. 2000;96: 1879–1888.
6. Spivak-Kroizman T, Lemmon MA, Dikic I, Ladbury JE, Pinchasi D, Huang J, Jaye M, Crumley G, Schlessinger J, Lax I. Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. *Cell*. 1994;79:1015–1024.
7. Logeart D, Prigent-Richard S, Jozefonvicz J, Letourneur D. Fucans, sulfated polysaccharides extracted from brown seaweed, inhibit vascular smooth muscle cell proliferation, I: comparison with heparin for antiproliferative activity, binding and internalization. *Eur J Cell Biol*. 1997;74: 376–384.
8. Nardella A, Chaubet F, Boisson-Vidal C, Blondin C, Durand P, Jozefonvicz J. Anticoagulant low molecular weight fucans produced by radical process and ion exchange chromatography of high molecular weight fucans extracted from the brown seaweed *Ascophyllum nodosum*. *Carbohydr Res*. 1996;289:201–208.
9. Chevolot L, Foucault A, Chaubet F, Kervarec N, Sinquin C, Fisher AM, Boisson-Vidal C. Further data on the structure of brown seaweed fucans: relationships with anticoagulant activity. *Carbohydr Res*. 1999;319: 154–165.
10. Millet J, Jouault SC, Mauray S, Theveniaux J, Sternberg C, Boisson Vidal C, Fischer AM. Antithrombotic and anticoagulant activities of a low molecular weight fucoidan by the subcutaneous route. *Thromb Haemost*. 1999;81:391–395.
11. Stearns NA, Prigent-Richard S, Letourneur D, Castellot JJ. Synthesis and characterization of highly sensitive heparin probes for detection of heparin-binding proteins. *Anal Biochem*. 1997;247:348–356.
12. Battle T, Arnal JF, Challah M, Michel JB. Selective isolation of rat aortic wall layers and their cell types in culture: application to converting enzyme activity measurement. *Tissue Cell*. 1994;26:943–955.
13. Urien S. MicroPharm-K, a microcomputer interactive program for the analysis and simulation of pharmacokinetic processes. *Pharm Res*. 1995; 12:1225–1230.
14. Feldman LJ, Aguirre L, Ziol M, Bridou JP, Nevo N, Michel JB, Steg PG. Interleukin-10 inhibits intimal hyperplasia after angioplasty or stent implantation in hypercholesterolemic rabbits. *Circulation*. 2000;101: 908–916.
15. McCaffrey TA, Falcone DJ, Borth W, Brayton CF, Weksler BB. Fucoidan is a non-anticoagulant inhibitor of intimal hyperplasia. *Biochem Biophys Res Commun*. 1992;184:773–781.
16. Bult H. Restenosis. a challenge for pharmacology. *Trends Pharmacol Sci*. 2000;21:274–279.
17. Gimple LW, Gertz SD, Haber HL, Ragosta M, Powers ER, Roberts WC, Sarembock IJ. Effect of chronic subcutaneous or intramural administration of heparin on femoral artery restenosis after balloon angioplasty in hypercholesterolemic rabbits: a quantitative angiographic and histopathological study. *Circulation*. 1992;86:1536–1546.
18. Hanke H, Oberhoff M, Hanke S, Hassenstein S, Kamenz J, Schmid KM, Betz E, Karsch KR. Inhibition of cellular proliferation after experimental balloon angioplasty by low-molecular-weight heparin. *Circulation*. 1992; 85:1548–1556.
19. Lablanche JM, McFadden EP, Meneveau N, Lusson JR, Bertrand B, Metzger JP, Legrand V, Grollier G, Macaya C, de Bruyne B, Vahanian A, Grentzinger A, Masquet C, Wolf JE, Tobelem G, Fontecave S, Vacheron A, d'Azemar P, Bertrand ME. Effect of nadroparin, a low-molecular-weight heparin, on clinical and angiographic restenosis after coronary balloon angioplasty: the FACT study: Fraxiparine Angioplastie Coronaire Transluminale. *Circulation*. 1997;96:3396–3402.
20. Zhang XW, Liu Q, Thorlacius H. Inhibition of selectin function and leukocyte rolling protects against dextran sodium sulfate-induced murine colitis. *Scand J Gastroenterol*. 2001;36:270–275.
21. Bojakowski K, Abramczyk P, Bojakowska M, Zwolinska A, Przybylski J, Gaciong Z. Fucoidan improves the renal blood flow in the early stage of renal ischemia/reperfusion injury in the rat. *J Physiol Pharmacol*. 2001; 52:137–143.
22. Shimaoka M, Ikeda M, Iida T, Taenaka N, Yoshiya I, Honda T. Fucoidin, a potent inhibitor of leukocyte rolling, prevents neutrophil influx into phorbol-ester-induced inflammatory sites in rabbit lungs. *Am J Respir Crit Care Med*. 1996;153:307–311.
23. Religa P, Kazi M, Thyberg J, Gaciong Z, Swedenborg J, Hedin U. Fucoidan inhibits smooth muscle cell proliferation and reduces mitogen-

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activated protein kinase activity. *Eur J Vasc Endovasc Surg*. 2000;20: 419–426.
24. Patel MK, Mulloy B, Gallagher KL, O'Brien L, Hughes AD. The antimitogenic action of the sulfated polysaccharide fucoidan differs from heparin in human vascular smooth muscle cells. *Thromb Haemost*. 2002; 87:149–154.
25. Logeart D, Prigent-Richard S, Boisson-Vidal C, Chaubet F, Durand P, Jozefonvicz J, Letourneur D. Fucans, sulfated polysaccharides extracted from brown seaweed, inhibit vascular smooth muscle cell proliferation, II: degradation and molecular weight effect. *Eur J Cell Biol*. 1997;74: 385–390.
26. Letourneur D, Caleb BL, Castellot JJ. Heparin binding, internalization, and metabolism in vascular smooth muscle cells, II: degradation and secretion in sensitive and resistant cells. *J Cell Physiol*. 1995;165: 687–695.
27. McCaffrey TA, Falcone DJ, Vicente D, Du B, Consigli S, Borth W. Protection of transforming growth factor-beta 1 activity by heparin and fucoidan. *J Cell Physiol*. 1994;159:51–59.
28. Giraux JL, Matou S, Bros A, Tapon-Bretaudiere J, Letourneur D, Fischer AM. Modulation of human endothelial cell proliferation and migration by fucoidan and heparin. *Eur J Cell Biol*. 1998;77:352–359.
29. Giraux JL, Tapon-Bretaudiere J, Matou S, Fischer AM. Fucoidan, as heparin, induces tissue factor pathway inhibitor release from cultured human endothelial cells. *Thromb Haemost*. 1998;80:692–695.
30. Hirsh J, Anand SS, Halperin JL, Fuster V. Mechanism of action and pharmacology of unfractionated heparin. *Arterioscler Thromb Vasc Biol*. 2001;21:1094–1096.
31. Heinzelmann M, Polk HC Jr, Miller FN. Modulation of lipopolysaccharide-induced monocyte activation by heparin-binding protein and fucoidan. *Infect Immun*. 1998;66:5842–5847.
32. Rogers C, Welt FG, Karnovsky MJ, Edelman ER. Monocyte recruitment and neointimal hyperplasia in rabbits: coupled inhibitory effects of heparin. *Arterioscler Thromb Vasc Biol*. 1996;16:1312–1318.
33. Chauvet P, Bienvenu JG, Theoret JF, Latour JG, Merhi Y. Inhibition of platelet-neutrophil interactions by fucoidan reduces adhesion and vasoconstriction after acute arterial injury by angioplasty in pigs. *J Cardiovasc Pharmacol*. 1999;34:597–603.
34. Linnemann G, Reinhart K, Parade U, Philipp A, Pfister W, Straube E, Karzai W. The effects of inhibiting leukocyte migration with fucoidin in a rat peritonitis model. *Intensive Care Med*. 2000;26:1540–1546.
35. Anastase-Ravion S, Carreno M, Blondin C, Ravion O, Champion J, Chaubet F, Haeffner-Cavaillon N, Letourneur D. Heparin-like polymers modulate proinflammatory cytokine production by lipopolysaccharide-stimulated human monocytes. *J Biomed Mater Res*. 2002;60:375–383.
36. Deux JF, Prigent-Richard S, D'Angelo G, Feldman LJ, Puvion E, Logeart-Avramoglou D, Pelle A, Boudghene FP, Michel JB, Letourneur D. A chemically modified dextran inhibits smooth muscle cell growth in vitro and intimal in stent hyperplasia in vivo. *J Vasc Surg*. 2002;35: 973–981.
37. Pereira MS, Mulloy B, Mourao PA. Structure and anticoagulant activity of sulfated fucans: comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. *J Biol Chem*. 1999;274:7656–7667.
38. Frenette PS, Weiss L. Sulfated glycans induce rapid hematopoietic progenitor cell mobilization: evidence for selectin-dependent and independent mechanisms. *Blood*. 2000;96:2460–2468.
39. Sweeney EA, Lortat-Jacob H, Priestley GV, Nakamoto B, Papayannopoulou T. Sulfated polysaccharides increase plasma levels of SDF-1 in monkeys and mice: involvement in mobilization of stem/progenitor cells. *Blood*. 2002;99: 44–51.
40. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:964–967.
41. Heldman AW, Cheng L, Jenkins GM, Heller PF, Kim DW, Ware M Jr, Nater C, Hruban RH, Rezai B, Abella BS, Bunge KE, Kinsella JL, Sollott SJ, Lakatta EG, Brinker JA, Hunter WL, Froehlich JP. Paclitaxel stent coating inhibits neointimal hyperplasia at 4 weeks in a porcine model of coronary restenosis. *Circulation*. 2001;103:2289–2295.
42. Sousa JE, Costa MA, Abizaid AC, Rensing BJ, Abizaid AS, Tanajura LF, Kozuma K, Van Langenhove G, Sousa AG, Falotico R, Jaeger J, Popma JJ, Serruys PW. Sustained suppression of neointimal proliferation by sirolimus-eluting stents: one-year angiographic and intravascular ultrasound follow-up. *Circulation*. 2001;104:2007–2011.

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