

Comparative Studies on the Mechanisms of Action of Four Polysaccharides on Arterial Restenosis

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Received 29 October 2007; accepted 10 April 2008

Abstract

Percutaneous coronary interventions play a major role in the management of patients affected by coronary artery diseases. However, their efficiency is impaired by restenosis, defined as a reduction of the vessel lumen, occurring a few months after the procedure. A low-molecular-weight fraction of fucoidan, a vegetal heparin-like sulphated polysaccharide, was recently shown to greatly reduce in-stent restenosis after angioplasty in rabbits. To better understand the *in vivo* anti-restenotic effects of this polymer, we used fractions of fucoidan and compared to heparin and dextran of different sizes. We carried out *in vitro* growth inhibition experiments on vascular smooth muscle cells, performed an *in vivo* pharmacokinetic study, and locally delivered fluorescently-labeled polysaccharides in rabbit iliac arteries after angioplasty with a non-occlusive catheter. The results indicated that (i) preparation of well-characterized fractions from natural fucoidan is compulsory for *in vitro* and *in vivo* studies, (ii) antiproliferative activity of sulphated polysaccharides on cultured smooth muscle cells is not a major predictive factor for the reduction of restenosis *in vivo* and (iii) pharmacokinetic parameters and binding of low-molecular-weight fucoidan on angioplasty-induced injured vascular walls are important local and general factors controlling its mechanisms of action.

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Keywords

Fucoidan, heparin, polysaccharides, pharmacokinetics, restenosis, vascular smooth muscle cells

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1. Introduction

Balloon angioplasty, also called percutaneous transluminal coronary angioplasty, revolutionized the surgical treatment of coronary artery disease. However, despite increased surgical experience, a clinical failure phenomenon called restenosis occurred in 30–50% of patients undergoing a simple balloon angioplasty and 10–30% of patients who receive an intravascular stent [1]. This process is a response to the injury and promotes intimal hyperplasia by eliciting immune and vascular smooth cell proliferation response.

Treatment strategies use two major approaches, one consisting of attempting to improve haemocompatibility and biocompatibility of stents by modifying their surfaces with less thrombogenic and inflammatory materials, including polymers, and the other is the delivery of pharmacologic anti-proliferative agents by systemic or by local drug delivery *via* drug-eluting stents. Despite promising results in the short term, recent studies reported increased rates of late stents thrombosis with drug-eluting stents [2, 3].

Polysaccharides constitute a large family of polymers which interact with the arterial wall. Natural or synthetic polysaccharides, such as heparin, derivative dextrans or low-molecular-weight (LMW) fucoidan, display pleiotrophic effects such as growth factor protection [4], endothelial cell proliferation [5] and vascular smooth muscle cell inhibition [6–9].

In contrast to unfractionated heparin used in clinical practice, LMW fucoidan is devoid of anti-coagulant activity and may represent an interesting therapeutic agent. Used in an *in vivo* model of restenosis, this polysaccharide reduced intimal hyperplasia and increased luminal cross-sectional area [7, 10]. Knowing these properties, it was important to better understand the systemic and local effects of this type of potential macromolecular drug. For that purpose, we investigated here the effect of four polysaccharides, two obtained by chemical separation (LMW fucoidan and the fractionated fucoidan) and the other two (heparin and dextran) were commercially available, on vascular muscle cell inhibition, pharmacokinetics and *in vivo* local drug delivery with a new non-occlusive catheter.

2. Materials and Methods

2.1. Polysaccharide Preparation

Fractions of fucoidan (fractionated fucoidan) with intermediate molecular weight (M_w) or low molecular weight (LMW fucoidan) were isolated from high-molecular-weight extracts of brown marine algae. Fractionated fucoidan was obtained by size-exclusion chromatography [11] and LMW fucoidan was obtained by radical depolymerisation as previously described [12]. Unfractionated hog intestine heparin was obtained from Sanofi Recherche (Centre Choay, Gentilly/France). Native 40 kDa dextran, used as a control neutral polysaccharide, was purchased by Amersham Pharmacia Biosciences. LMW dextran (approx. 6 kDa) was purchased

from Sigma. Fractionated fucoidan, LMW fucoidan and heparin were fluorolabeled using 5-([4,6-dichlorotriazin-2-yl]amino) fluorescein (DTAF) [13, 14]. Fluorolabeled FITC 40 kDa dextran was commercially available (Sigma).

2.2. Polysaccharide Characterisation

Size-exclusion chromatography was performed at 37°C on two serially connected columns protected by a guard column (Shodex OH-Pak SB-G, SB-804HQ and SB-803HQ). GPC-Max chromatographic system from Viscotek (Basingstoke, UK) used a triple detection by an original system including low- (7°) and right-angle laser light scattering at $\lambda = 633$ nm, refractive index measurement and differential viscometric detection. A degassed 0.05 M phosphate saline buffer (with 0.15 M NaCl, pH 7.4) was used after filtration on a 0.22 μm pore size membrane (Dura-pore, Millipore). The flow rate was 0.5 ml/min. Each sample was dissolved in the buffer at final concentration of 8 mg/ml and 100 μl was injected. Refractive index increments were fixed to 0.132 ml/mg for heparin, LMW fucoidan and unfractionated fucoidan, or to 0.147 ml/mg for dextran. Molecular weights (number-average molecular weight (M_n) and M_w) and hydrodynamic radii (R_h) were determined using a combination of light-scattering, viscometric and refractive index measurements. The polydispersity index was equal to the M_n/M_w ratio.

2.3. Cell Culture

SMCs from Sprague–Dawley rat thoracic aorta were isolated from media explants as previously described [15]. Cells were cultured in Eagle's Minimum Essential Medium (MEM, Sigma) supplemented with 10% fetal calf serum (FCS) (Biowhitaker) and 2 mM L-glutamine (Sigma) at 37°C in 5% CO₂. Cultures from two to four passages were used throughout this work. The nature of SMCs was confirmed by morphological examination and by positive immunofluorescence for smooth muscle cell α -actin [10].

2.4. In Vitro SMC Growth Inhibition Studies

Vascular SMCs (1.2×10^4 cells) were plated into 24-well plates in MEM containing 10% FCS. After 24 h, cells were growth-arrested by culture in medium with 0.4% serum for 72 h. Cell growth was initiated by medium change (MEM + 10% FCS) with or without increasing concentrations (from 10^{-4} to 10^2 $\mu\text{mol/l}$) of polysaccharides. At day 5, cells were harvested with trypsin-EDTA and counted using a Coulter Counter ZM. All experiments were performed at least twice and in quadruplicate samples. The net growth of SMCs was obtained by taking off the starting cell number from the cell number at the end of the experiment. Percent inhibition was calculated as described [9] using the formula: Inhibition = $(1 - (\text{net growth in the presence of polysaccharides}/\text{net growth in controls})) \times 100$. The concentration that inhibited cell growth by 50% (IC₅₀) was determined from log dose–response curves [16].

2.5. Pharmacokinetic Experiments

Adult male Wistar rats (Wi/Wi, Ico, Ifacredo, France) weighing 280–300 g were used for this study. Animal protocols were approved by Bichat Hospital Institutional Animal Care and Use Committee. Animals were anaesthetized using intraperitoneal sodium pentobarbital solution (0.1 ml/kg). A silicon-tipped polyethylene catheter was inserted into the right jugular vein. A bolus of 5 mg/kg of fluorescent fractionated fucoidan ($n = 5$), LMW fucoidan ($n = 6$), heparin ($n = 5$), or neutral dextran ($n = 5$) was injected intravenously *via* the jugular vein catheter followed by saline solution to flush the catheter. Blood samples were obtained from the catheter at 0, 15 and 30 min and 1, 2, 4 and 6 h. After blood centrifugation, the concentration of fluorescent polysaccharides was measured in the plasma by a spectrofluorimeter (F-2000, Hitachi, Japan) at $\lambda_{\text{excitation}}$ and $\lambda_{\text{emission}}$ of 489 and 515 nm, respectively. A calibration curve was performed for each fluorescent polysaccharide.

2.6. Pharmacokinetic Analysis

Equation (1) describes the time-course of polysaccharide concentration in the blood according to a two-compartment open model (1):

$$Ct = Ae^{-\alpha t} + Be^{-\beta t}, \quad (1)$$

where A and B are constants. Parameters were determined using the non-linear least-squares program Micropharm[®] [17]. The elimination half-time period, $T_{1/2\beta}$ (β phase), is the time with the end of which the concentration of drug in plasma decreases by half. It was calculated from the value of the slope (β in (2)):

$$T_{1/2\beta} = \log 2 / \beta, \quad (2)$$

The clearance (Cl) corresponding to the elimination of the drug in ml/min is described by the equation: $Cl = AUC/D$ where AUC stands for the area under the curve. Clearance was calculated using the Micropharm[®] trapezoidal rule [17]. D is the administered dose of polysaccharide. The apparent distribution volume (V_d) is the ratio of the amount of drug in a whole organism and its plasma concentration measured at the same time.

2.7. Local Carotid Drug Delivery with a Non-occlusive Catheter

Animal model were performed in a male New Zealand White rabbits (Animal Production Centre, Janvier, Orleans, France) in order to use a non-occlusive catheter (Dispatch[®], Nycomed, France) which was design for human utilisation [18]. Animals weighing 3.5 kg were anaesthetized with intravenous sodium pentobarbital solution and the right carotid artery was catheterized with a 5F sheath (Terumo, Japan). A 3.0-mm-diameter, 20-mm-long balloon dilatation catheter was advanced over a standard 0.4 mm flexible wire in right iliac artery. The iliac artery was in-

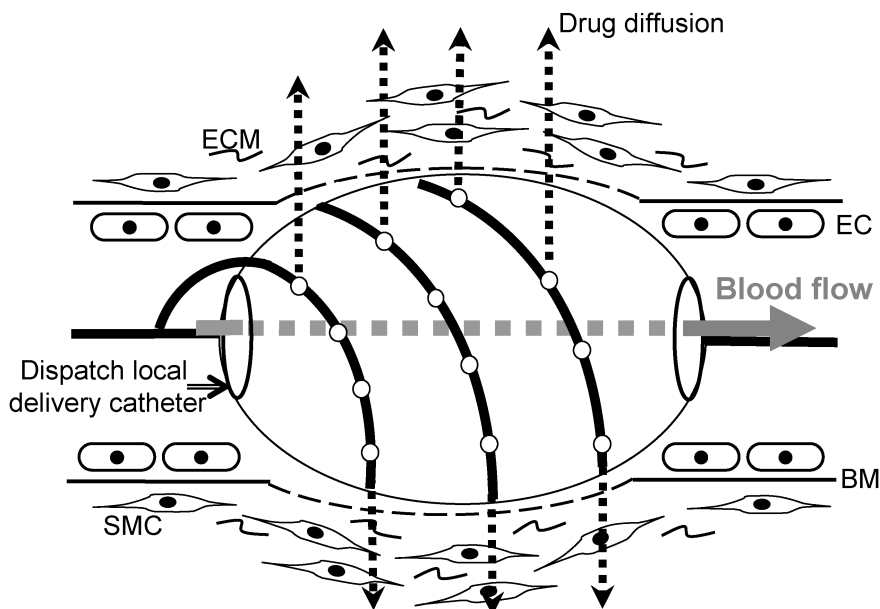


Figure 1. Local delivery of fluorescent polysaccharides in ballooned iliac artery using a non-occlusive dispatch catheter. After angioplasty, the basement membrane was disrupted (interrupted line) and endothelial cells were damaged. Dashed arrows indicate polysaccharide diffusion (5 mg/ml) in vessel wall through holes in the circular catheter running around the balloon. Central lumen present in dispatch catheter maintained the blood flow in the iliac artery during the procedure. Central arrow in grey indicates blood flow. EC, endothelial cell layer; BM, basement membrane; SMC, smooth muscle cells; ECM, extracellular matrix.

jured by three successive 1-min over-inflations at 10 atm with a 1-min reflow after each inflation. The balloon dilatation catheter was removed and a 20-mm-long non-occlusive dispatch local delivery catheter (Nycomed, Paris, France) was used to deliver the fluorescent polysaccharides after angioplasty. This catheter allows local delivery of drug while maintaining blood flow in the artery (Fig. 1). One ml of fluorescent polysaccharides at 5 mg/ml (LMW fucoidan, heparin or dextran) was perfused during 5 min while keeping the blood flow. Damaged segment and untreated adjacent segments were excised after local delivery and examined en face under fluorescent microscopy (Carl Zeiss, Germany) and photographed using Q Capture Pro Software (Qimaging, Canada).

2.8. Statistics

Results are expressed as means \pm SD. The significance of the differences between groups was analyzed by one-way analysis of variance (ANOVA) and a Scheffé test for selected comparisons between groups. Values of $P < 0.05$ were considered significant.

Table 1.

Physico-chemical properties of polysaccharides used in this study

Polysaccharide	Source	Molecular mass (kDa)	Polydispersity index ($I = M_n/M_w$)	R_h (nm)
Unfractionated fucoidan	Commercial	34.7	2.1	5.8
Native 40 kDa dextran	Commercial	40.8	1.2	5.5
Fractionated fucoidan	Size fractionation	19.0	1.8	3.9
Heparin	Commercial	11.8	1.2	3.4
LMW fucoidan	Radical depolymerisation	6.9	1.5	2.3
LMW dextran	Commercial	6.8	1.2	2.3

Parameters were obtained by triple detection using low- (7°) and right-angle laser light scattering, refractive index and differential viscometry in physiological phosphate buffer at 8 mg/ml concentration for all polysaccharides. M_n , number-average molecular weight, M_w , molecular weight; R_h , hydrodynamic radius.

3. Results

3.1. Physico-Chemical Characterisations of Polysaccharides

Two families of sulphated polysaccharides, heparin and fucoidan, have been characterized and compared to neutral dextran, in order to study the influence on restenosis of both chemical structure and molecular mass. Molecular mass, polydispersity index and hydrodynamic radii are reported in Table 1 for each polysaccharide. They were determined in physiological buffer at 37°C using a triple detection coupled to a chromatographic system that allows an absolute determination of molecular masses. Three ranges of molecular mass were evidenced: native dextran and unfractionated fucoidan (molecular mass >30 kDa), fractionated fucoidan and heparin (12–19 kDa) and LMW fucoidan or dextran (6–7 kDa). Hydrodynamic radii were correlated with molecular mass for the polysaccharides, i.e., 5–6 nm for HMW polysaccharides, 3–4 nm for the second group of polymers and around 2.5 nm for LMW fucoidan or LMW dextran. These values allowed further crossed comparisons of biological activities of the studied samples.

3.2. In Vitro SMC Growth Inhibition by Polysaccharides

The growth of vascular SMCs cultured for 5 days was evaluated by direct cell counting. The presence of polysaccharides in culture medium affected the SMC growth in different fashions (Fig. 2). On a molar basis, fractionated fucoidan is the most potent anti-proliferative polysaccharide on SMC growth with an IC_{50} at $0.07 \mu\text{mol/l}$, compared to $0.4 \mu\text{mol/l}$ for heparin and $4 \mu\text{mol/l}$ for LMW fucoidan. Native dextran was inactive on SMC growth even at high concentrations (Fig. 2).

3.3. Plasma Concentrations of Polysaccharides

Plasma concentration–time profiles of heparin, fractionated fucoidan, LMW fucoidan and native dextran are shown in Fig. 3. The pharmacokinetic behaviour

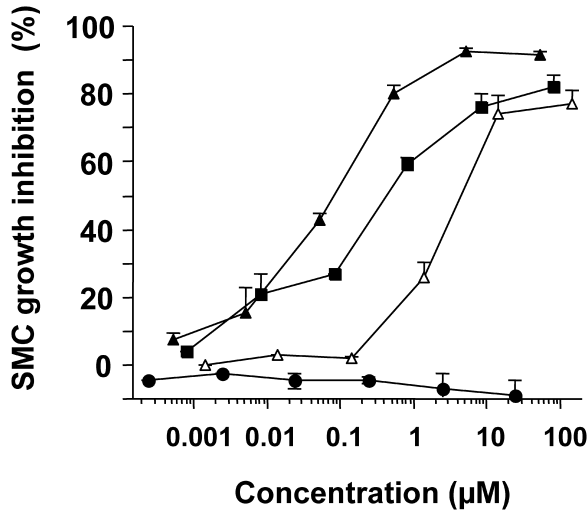


Figure 2. Vascular SMC growth inhibition *versus* polysaccharide concentration. Growth-arrested cells were released from quiescent G0 phase by addition of culture medium containing 10% serum in presence of increasing concentrations of fractionated fucoidan (▲), LMW fucoidan (△), heparin (■) or native dextran (●). The cell number at day 5 was assessed as described in Materials and methods and values of SMC growth inhibition were expressed as means \pm SD of 3 experiments.

of polysaccharides is better described using a two-compartment model, indicating rapid distribution and slow elimination phases for the compounds. Pharmacokinetic parameters are summarized in Fig. 4. Data showed that clearance (Fig. 4A) and distribution volume (Fig. 4B) for LMW fucoidan were significantly lower ($P < 0.001$) than for fractionated fucoidan and heparin. In addition, the half-life period (Fig. 4C) of LMW fucoidan was similar to heparin and significantly ($P < 0.005$) lower than fractionated fucoidan. We also showed that clearance, half-life period and distribution volume of native dextran used as a control were significantly ($P < 0.005$) the lowest compared to the other tested polysaccharides (Fig. 4).

3.4. Local Delivery of Polysaccharides in Injured Artery

We used a non-occlusive dispatch catheter to locally deliver the polysaccharides into injured rabbit aorta. Fluorescent compounds were injected into the angioplasty-induced injured vascular walls of the iliac artery (4–5 mm) while maintaining the blood flow (Fig. 1). After injection of fluorescent polysaccharides, untreated adjacent iliac segments revealed no fluorescence (Fig. 5A). No significant fluorescence was detected after local delivery of fluorescent dextran (Fig. 5B). As previously reported by others [19], microscopic study revealed fluorescence on the damaged arteries after local delivery of fluorescent heparin (Fig. 5C). Interestingly, we observed here that fluorescent LMW fucoidan also bind tightly to the injured vessel walls *in vivo* (Fig. 5D).

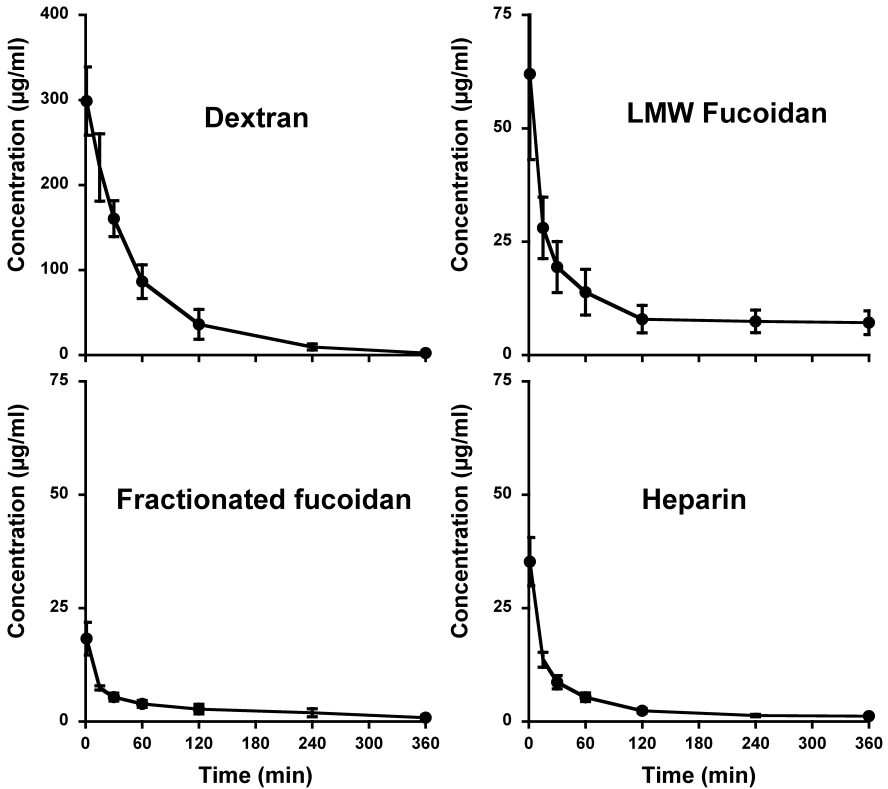


Figure 3. Plasmatic rates of fluorescent polysaccharides after intravenous injection (5 mg/kg) of heparin, fractionated fucoidan, LMW fucoidan and dextran. Plasma concentration is measured at the indicated times by spectrofluorescence. A calibration curve was performed for each fluorescent polysaccharide. Values are expressed as means \pm SD ($n = 5-7$ rats).

4. Discussion

Polysaccharides are frequently used in the prevention of thrombosis or post-stent implantation restenosis [20, 21]. Recently, we reported very promising *in vivo* results in the prevention of post-stenting restenosis after balloon angioplasty in rabbit using LMW fucoidan heparin-like sulphated polysaccharides devoid of anticoagulant activity [7, 10], and devoid of risk of infection by a non-mammalian origin. Here, we present data about the physical characterisation and some possible mechanisms of action of LMW fucoidan in comparison to heparin.

One of the major questions which arise is the comparison for polysaccharides of the results obtained on *in vitro* or *in vivo* effects (wound healing, angiogenesis, restenosis). Indeed, the molecular characteristics of polysaccharides have to be known to compare their effects. Our results indicated that the size of commercial or clinical grade polysaccharides can be presented in three distinct categories ($R_h < 2$ nm; 3–4 nm and >4 nm) and the molecular masses could be compared two by two. The determination of polydispersity limited the experimental use of the

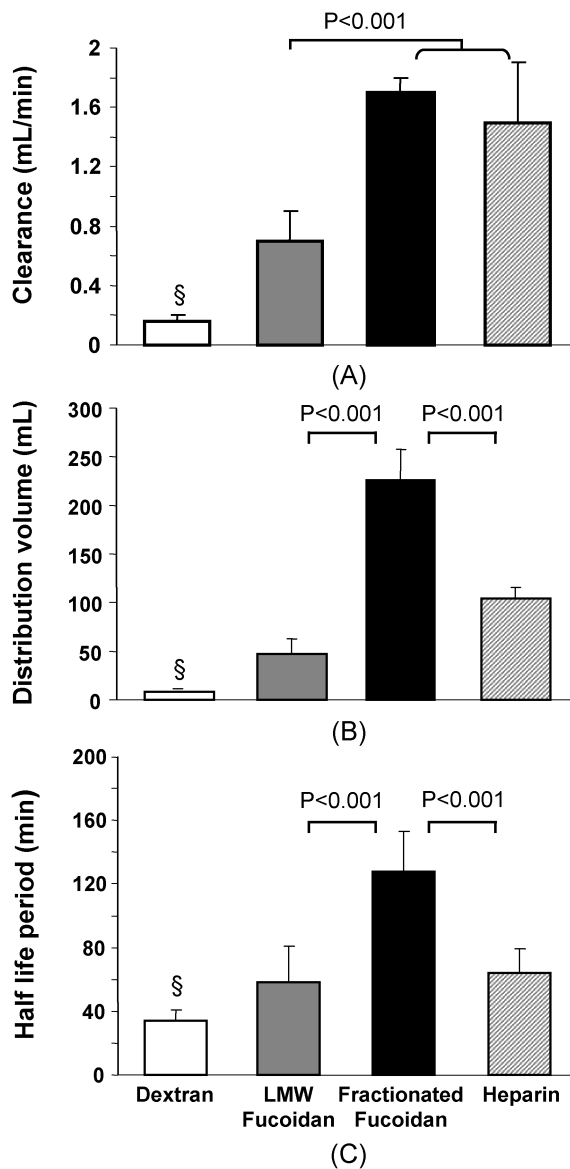


Figure 4. Pharmacokinetic parameters after fluorescent polysaccharide intravenous injection in rats. Pharmacokinetic parameters were analyzed using the non-linear least-squares program Micropharm[®] with a two-compartment model. Clearance (A), distribution volume (B) and half-life period (C) are reported for dextran, LMW fucoïdan (LMWF), fractionated fucoïdan and heparin. Results are expressed as means \pm SD ($n = 5-7$). § $P < 0.005$ for dextran compared to LMW fucoïdan, fractionated fucoïdan and heparin.

commercially available fucoïdan. This widely-used polysaccharide [22–24] with a high polydispersity (>2) is very heterogeneous and highly variable from batch to batch (values of 34, 55, 66 and 193 kDa were obtained for four different batches

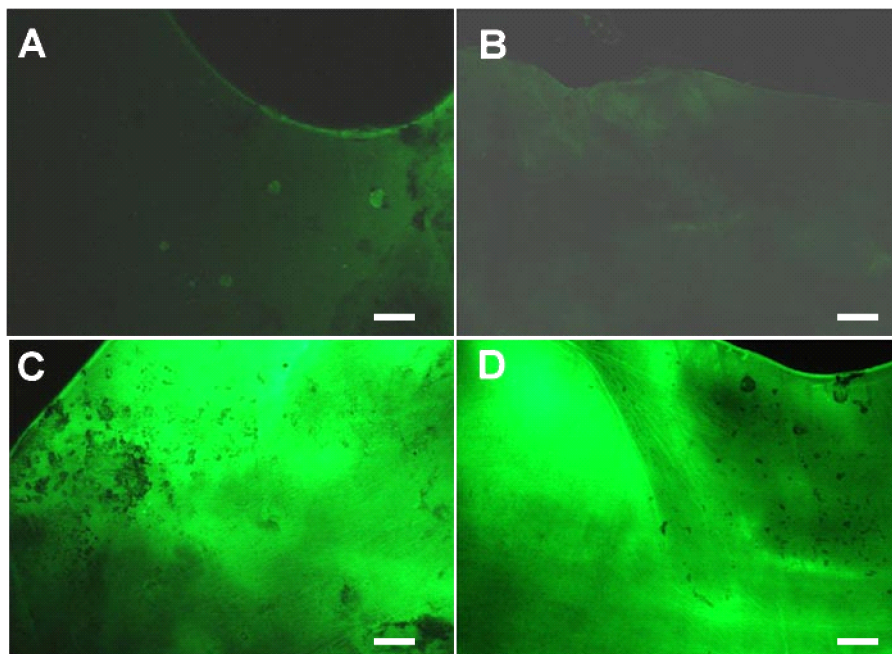


Figure 5. *En face* fluorescent microscopic pictures of the untreated adjacent rabbit iliac artery (A) and the ballooned artery after 5 min local delivery of 5 mg/ml of polysaccharides (B, C, D). Untreated segment after injection of polysaccharides revealed no fluorescence (A). No significant fluorescence of the damaged arterial wall was detected after local delivery of fluorescent dextran (B). Fluorescent examination revealed fluorescence of the injured arterial wall after local delivery of fluorescent heparin (C), or LMW fucoidan (D). Scale bars = 0.5 cm. This figure in published in colour at <http://www.ingenta.com>

from the same provider). Consequently, it is difficult to compare the effects obtained by different groups with this polymer to clinical 40 kDa dextran. For these reasons, the commercial fucoidan was not used further in the study. Fucoidan was, therefore, fractionated [11, 12] and compared to clinical grade heparin and dextran. Determination of the polydispersity index of fucoidan fractions, heparin and LMW fucoidan evidenced more homogeneous polymers ($I = 1.8, 1.2$ and 1.5 , respectively); therefore, these compounds were used in this study and compared to a neutral commercial dextran ($I = 1.2$).

From cell-culture experiments, it is noteworthy that inhibition of SMC growth was molecular-mass-dependent for the studied polysaccharides. IC_{50} values were 0.07 and $4 \mu\text{mol/l}$, respectively, for fractionated fucoidan (19 kDa) and LMW fucoidan (7 kDa). For unfractionated heparin (12 kDa), the IC_{50} value was $0.4 \mu\text{mol/l}$ and $>100 \mu\text{mol/l}$ for LMW heparin [10]. Other studies showed a relationship between size and charge of various polysaccharides or glycosaminoglycans (heparin [25, 26], oligosaccharide fragments [27], hyaluronan [28, 29], chondroitin sulphate, dermatan sulphate [30]) and vascular smooth muscle cell growth inhibition.

In our *in vitro* growth studies we also observed that the anti-proliferative effect of LMW fucoidan remains one order of magnitude below the anti-proliferative level of unfractionated heparin. In contrast, we have reported opposite results *in vivo* for the prevention of restenosis, LMW fucoidan being a potent anti-restenotic agent that was superior to heparin [7, 10]. LMW fucoidan, a weaker antiproliferative agent on *in vitro* SMC growth than a functionalized dextran (IC_{50} of 1.6 $\mu\text{mol/l}$ for functionalized dextran vs. 4 $\mu\text{mol/l}$ for LMW fucoidan), is a better anti-restenotic agent *in vivo* after angioplasty and stenting [7, 10].

Taken together, our data evidenced that *in vitro* anti-proliferative effect of the sulphated polysaccharides on cultured SMCs is not the major predictive parameter in the screening of such polysaccharides for the treatment of *in vivo* restenosis. Indeed, removing vascular smooth muscle cells from their native environment for *in vitro* studies altered this response and could explain some of the observed differences. Vascular SMC function is influenced by a wide variety of biochemical factors and mechanical signals involved in the normal cell function [31]. Stegemann and Nerem observed that extracellular matrix (ECM) components or heparin modulate the function of the smooth muscle cell *in vitro* and the results observed in a two-dimensional system do not occur systematically in a three-dimensional system [31]. However, the only experimental model which takes into account the cellular environment, the ECM, the biochemical-secreted factors and the mechanical forces, is the animal model that presents the advantage of an integrated physiology.

Sulphated polysaccharides exhibited plasmatic behaviour that may greatly influence their activity on restenosis. Pharmacokinetics of polysaccharides showed a bi-exponential decline phase with an initial fast decrease and a second almost linear phase. The first phase includes the distribution of sulphated polysaccharides from the plasma in a second compartment (tissue). This second compartment is likely the liver, the main way of elimination reported for anionic dextrans [32, 33]. We also noticed marked differences between pharmacokinetic parameters of LMW fucoidan on one hand, and fractionated fucoidan and heparin on the other hand. Fractionated fucoidan and heparin exhibited important distribution volume and diffuse markedly in the extravascular compartment, in contrast to LMW fucoidan that diffuses less in the body and is more confined in the vascular compartment. These results could explain the low efficacy of heparin and LMW heparin at the local site and the high concentration of heparin (300 $\mu\text{g/ml}$) required to inhibit intimal hyperplasia in an *in vivo* model. It is unlikely that such concentrations could be reached in humans [34]. Thus, LMW fucoidan that is more in contact with vascular walls may have the highest potential to bind vascular cells and proteins accessible in the remodelling process involved in restenosis.

We also evidenced the binding of charged polysaccharides (heparin and LMW fucoidan) after local *in vivo* delivery to damaged vascular walls. Mechanical injury induced by angioplasty disrupts the endothelial barrier and exposes extracellular matrix and SMCs to blood (Fig. 1). Circulating heparin-like sulphated polysaccharides can bind to heparin-binding domains of ECM proteins. As reported for heparin

or other polyaromatic heparin-mimicking compounds [19, 35–37], the binding of heparin-like polymers could locally modulate the bioavailability of key regulatory components such as heparin-binding growth factors present in the ECM [38–40]. Similarly to synthetic polysaccharides that exhibited *in vivo* affinity for damaged muscular tissue after systemic injection [4], the increasing concentration of LMW fucoidan at the damaged vascular wall may lead to reduction of the restenosis rate. Other properties of LMW fucoidan such as antithrombotic [41, 42], pro-angiogenic [43] and immunomodulatory effects on human blood cells [44] may also play a significant role in prevention of restenosis. The influence on restenosis of these properties for LMW fucoidan is currently under investigation with appropriate *in vitro*, *ex vivo* and *in vivo* experiments.

5. Conclusions

The data reported here suggest that anti-proliferative activity of polysaccharides on cultured SMC growth is not a major index for the screening of such drugs for restenosis. The favourable pharmacokinetic behaviour and the local affinity for damaged vascular wall probably contribute to a great extent to modulate *in vivo* various effects in this multi-factorial pathological process. These parameters are important to understand the potent inhibitory action of such polymers on restenosis before clinical studies.

Acknowledgements

This work was financially supported by INSERM, the Fondation Bettencourt-Schueller (Prix Coup d'Élan), the Fondation de l'Avenir, the Fondation de la Recherche Médicale, the Ministère de la Recherche and Université Paris 13 (BQR and IFR Paris Nord). The authors thank L. Louedec (INSERM U 698, Paris) for her excellent technical assistance.

References

1. R. Fattori and T. Piva, *Lancet* **361**, 247 (2003).
2. J. Daemen, P. Wenaweser, K. Tsuchida, L. Abrecht, S. Vaina, C. Morger, N. Kukreja, P. Juni, G. Sianos, G. Hellige, R. T. van Domburg, O. M. Hess, E. Boersma, B. Meier, S. Windecker and P. W. Serruys, *Lancet* **369**, 667 (2007).
3. C. Stettler, S. Wandel, S. Allemann, A. Kastrati, M. C. Morice, A. Schomig, M. E. Pfisterer, G. W. Stone, M. B. Leon, J. S. de Lezo, J. J. Goy, S. J. Park, M. Sabate, M. J. Suttorp, H. Kelbaek, C. Spaulding, M. Menichelli, P. Vermeersch, M. T. Dirksen, P. Cervinka, A. S. Petronio, A. J. Nordmann, P. Diem, B. Meier, M. Zwahlen, S. Reichenbach, S. Trelle, S. Windecker and P. Juni, *Lancet* **370**, 937 (2007).
4. A. Meddahi, F. Bree, D. Papy-Garcia, J. Gautron, D. Barritault and J. P. Caruelle, *J. Biomed. Mater. Res.* **62**, 525 (2002).
5. D. Letourneur, D. Machy, A. Pelle, E. Marcon-Bachari, G. D'Angelo, M. Vogel, F. Chaubet and J. B. Michel, *J. Biomed. Mater. Res.* **60**, 94 (2002).

6. D. Logeart, S. Prigent-Richard, C. Boisson-Vidal, F. Chaubet, P. Durand, J. Jozefonvicz and D. Letourneur, *Eur. J. Cell Biol.* **74**, 385 (1997).
7. J. F. Deux, S. Prigent-Richard, G. d'Angelo, L. J. Feldman, E. Puvion, D. Logeart-Avramoglou, A. Pelle, F. P. Boudghene, J. B. Michel and D. Letourneur, *J. Vasc. Surg.* **35**, 973 (2002).
8. J. J. Castellot Jr., D. L. Cochran and M. J. Karnovsky, *J. Cell Physiol.* **124**, 21 (1985).
9. D. Letourneur, B. L. Caleb and J. J. Castellot Jr., *J. Cell Physiol.* **165**, 676 (1995).
10. J. F. Deux, A. Meddahi-Pelle, A. F. Le Blanche, L. J. Feldman, S. Collicec-Jouault, F. Bree, F. Boudghene, J. B. Michel and D. Letourneur, *Arterioscler. Thromb. Vasc. Biol.* **22**, 1604 (2002).
11. C. Blondin, F. Chaubet, A. Nardella, C. Sinquin and J. Jozefonvicz, *Biomaterials* **17**, 597 (1996).
12. A. Nardella, F. Chaubet, C. Boisson-Vidal, C. Blondin, P. Durand and J. Jozefonvicz, *Carbohydr. Res.* **289**, 201 (1996).
13. D. Logeart, S. Prigent-Richard, J. Jozefonvicz and D. Letourneur, *Eur. J. Cell Biol.* **74**, 376 (1997).
14. N. A. Stearns, S. Prigent-Richard, D. Letourneur and J. J. Castellot Jr., *Anal. Biochem.* **247**, 348 (1997).
15. E. Battle, J. Arnal, M. Challah and J. B. Michel, *Tissue Cell* **26**, 943 (1997).
16. D. Letourneur, D. Logeart, T. Avramoglou and J. Jozefonvicz, *J. Biomater. Sci. Polymer Edn* **4**, 431 (1993).
17. S. Urien, *Pharm. Res.* **12**, 1225 (1995).
18. M. Mazighi, D. Tchetché, Y. Goueffic, A. San Juan, L. Louedec, D. Henin, J. B. Michel, M. P. Jacob and L. J. Feldman, *J. Vasc. Surg.* **44**, 1067 (2006).
19. M. A. Lovich and E. R. Edelman, *Circ. Res.* **77**, 1143 (1995).
20. R. L. Geary, N. Koyama, T. W. Wang, S. Vergel and A. W. Clowes, *Circulation* **91**, 2972 (1995).
21. L. W. Gimple, H. C. Herrmann, M. Winniford and E. Mammen, *Am. J. Cardiol.* **83**, 1524 (1999).
22. V. Kanabar, S. J. Hirst, B. J. O'Connor and C. P. Page, *Br. J. Pharmacol.* **146**, 370 (2005).
23. E. A. Sweeney, H. Lortat-Jacob, G. V. Priestley, B. Nakamoto and T. Papayannopoulou, *Blood* **99**, 44 (2002).
24. E. A. Sweeney, G. V. Priestley, B. Nakamoto, R. G. Collins, A. L. Beaudet and T. Papayannopoulou, *Proc. Natl. Acad. Sci. USA* **97**, 6544 (2000).
25. J. J. Castellot Jr., K. Wong, B. Herman, R. L. Hoover, D. F. Albertini, T. C. Wright, B. L. Caleb and M. J. Karnovsky, *J. Cell Physiol.* **124**, 13 (1985).
26. J. J. Castellot Jr., D. L. Beeler, R. D. Rosenberg and M. J. Karnovsky, *J. Cell Physiol.* **120**, 315 (1984).
27. T. C. Wright Jr., J. J. Castellot Jr., M. Petitou, J. C. Lormeau, J. Choay and M. J. Karnovsky, *J. Biol. Chem.* **264**, 1534 (1989).
28. B. Joddar and A. Ramamurthi, *Biomaterials* **27**, 2994 (2006).
29. A. Chajara, M. Raoudi, B. Delpech and H. Levesque, *Atherosclerosis* **171**, 15 (2003).
30. D. L. Cochran, J. J. Castellot Jr. and M. J. Karnovsky, *J. Cell Physiol.* **124**, 29 (1985).
31. J. P. Stegemann and R. M. Nerem, *Exp. Cell Res.* **283**, 146 (2003).
32. T. Yamaoka, M. Kuroda, Y. Tabata and Y. Ikada, *Int. J. Pharm.* **113**, 149 (1995).
33. J. F. Deux, A. F. Le Blanche, F. Boudghene, J. B. Michel and D. Letourneur, *Curr. Trends Polym. Sci.* **6**, 111 (2001).
34. M. A. Lovich and E. R. Edelman, *Proc. Natl. Acad. Sci. USA* **96**, 11111 (1999).
35. H. Araki, J. Muramoto, K. Nishi, M. Jougasaki and M. Inoue, *Circ. Res.* **71**, 577 (1992).
36. C. Blondin, I. Bataille and D. Letourneur, *Crit. Rev. Ther. Drug Carrier Syst.* **17**, 327 (2000).
37. B. Medalion, G. Merin, H. Aingorn, H. Q. Miao, A. Nagler, A. Elami, R. Ishai-Michaeli and I. Vlodavsky, *Circulation* **95**, 1853 (1997).

38. Y. Hamma-Kourbali, A. Starzec, R. Vassy, A. Martin, M. Kraemer, G. Perret and M. Crepin, *Br. J. Cancer* **89**, 215 (2003).
39. D. Logeart-Avramoglou, R. Huynh, F. Chaubet, L. Sedel and A. Meunier, *Biochem. Pharmacol.* **63**, 129 (2002).
40. K. Senni, C. Borchiellini, A. Duchesnay, B. Pellat, D. Letourneur and P. Kern, *J. Biomed. Mater. Res.* **40**, 164 (1998).
41. J. Millet, S. C. Jouault, S. Mauray, J. Theveniaux, C. Sternberg, C. Boisson Vidal and A. M. Fischer, *Thromb. Haemost.* **81**, 391 (1999).
42. J. L. Giraux, S. Matou, A. Bros, J. Tapon-Breaudiere, D. Letourneur and A. M. Fischer, *Eur. J. Cell Biol.* **77**, 352 (1998).
43. C. E. Luyt, A. Meddahi-Pelle, B. Ho-Tin-Noe, S. Collic-Jouault, J. Guezennec, L. Louedec, H. Prats, M. P. Jacob, M. Osborne-Pellegrin, D. Letourneur and J. B. Michel, *J. Pharmacol. Exp. Ther.* **305**, 24 (2003).
44. S. Anastase-Ravion, M. P. Carreno, C. Blondin, O. Ravion, J. Champion, F. Chaubet, N. Haeffner-Cavaillon and D. Letourneur, *J. Biomed. Mater. Res.* **60**, 375 (2002).