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Letter to the Editors-in-Chief

Low molecular weight fucoidan promotes FGF-2-induced vascular tube formation by human endothelial cells, with decreased PAI-1 release and ICAM-1 downregulation

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Dear Sir,

Basic fibroblast growth factor (FGF-2) is a member of the heparin-binding growth factor family involved in neovascularization (1). Compounds compatible with therapeutic use and able to modulate FGF-2 activity could be of interest for revascularization of ischemic areas. During neovascularization, endothelial cells first acquire invasive properties by secreting proteases that degrade the basal membrane (2). Endothelial cells synthesize and release fibrinolytic system proteases, such as tissue-type and urinary plasminogen activators (t-PA and u-PA respectively), as well as their physiological inhibitor, plasminogen activator inhibitor-1 (PAI-1). They also express urokinase receptor (uPAR) on their surface. Endothelial cells then migrate and differentiate, leading to the formation of new vascular tubes. These steps in angiogenesis alter the expression and/or activity of integrins and cellular adhesion molecules such as PECAM-1 and ICAM-1, allowing new cell-cell interactions and binding to ligands of the extracellular matrix (3, 4). In a previous study, we reported that a low molecular weight fucoidan (LMWF) of marine plant origin (brown algae *Ascophyllum nodosum*) potentiated FGF-2-induced tube formation by human endothelial cells from umbilical vein (HUVEC) through over-expression of the α6 integrin subunit (5). HUVEC, when stimulated with FGF-2 and a low molecular weight heparin (dalteparin), also formed tubes, though to a lesser extent (5). The purpose of the present study was to determine whether LMWF, as compared to dalteparin (concomitant with α6 overexpression) modulates the expression of cellular adhesion molecules and the release of proteins involved in pericellular lysis. Compared to untreated cells, HUVEC pretreated for 72 h with FGF-2 (5 ng/mL) differentiated in a partially organized capillary network (Figure 1) on a basal membrane substitute (Matrigel). Flow cytometry showed that ICAM-1, though expressed on untreated cells, was no longer detectable on HUVEC treated with FGF-2 (Figure 1). No change was apparent with respect to PECAM-1 labeling. As shown in Figure 1, HUVEC, when incubated simultaneously with FGF-2 and 10 µg/ml of LMWF or dalteparin, branched and formed tubes with closed areas, denoting intense proangiogenic activity that was more pronounced with LMWF. No further effect on ICAM-1 or PECAM-1 expression was detected after addition of polysaccharides to FGF-2. However, a direct relationship between FGF-2-induced angiogenesis and down-regulation of ICAM-1 on tumor-infiltrating endothelial cells has been reported (4). The absence of detectable ICAM-1 expression on HUVEC surface should potentiate cell migration and vascular tube formation on Matrigel.

The mechanism of the proangiogenic effect of LMWF and dalteparin was then explored further. An ELISA was used to quantify the antigen levels of PAI-1 and t-PA (Asserachrom t-PA and PAI-1, a generous gift from Stago, Gennevilliers, France) in cell supernatants cultured for 72 h with 10 µg/ml LMWF or dalteparin with or without 5 ng/ml FGF-2, and flow cytometry was used to study uPAR expression on HUVEC surface in the same batch of cells. Compared to untreated cells, the PAI-1 level decreased by 50% in the supernatants of HUVEC treated with FGF-2 alone. When LMWF was added to FGF-2, the PAI-1 level decreased to 14% ($p < 0.001$) of control value (untreated cells). In contrast to LMWF, no effect was observed by addition of dalteparin to FGF-2 as compared to FGF-2 alone (50% of control value). However, Schnaper et al. (2) and Xin et al. (6) have described an inverse relationship between PAI-1 level and angiogenesis *in vitro*. In our study, stimulation of HUVEC with LMWF or dalteparin and FGF-2 had no effect on t-PA release (data not shown). uPAR was not expressed on untreated HUVEC, but became detectable in the presence of FGF-2. No further increase was evidenced upon addition of polysaccharides (Figure 1).

The relationship between tube formation, ICAM-1 down-regulation and decreased PAI-1 release was confirmed in HUVEC treated with LMWF or dalteparin alone (without FGF-2). As shown in Figure 1, flow cytometry revealed an increase of ICAM-1 labeling on HUVEC treated with 10 µg/ml LMWF (174%; $p < 0.01$) or dalteparin (187%; $p < 0.01$) alone,

which could account in part for the lack of tube formation (Figure 1). Moreover, the level of PAI-1 in supernatants of HUVEC treated in this manner was not modified as compared to untreated cells. Thus, the PAI-1 levels (2-fold increase as compared to FGF-2-treated cells) could be related to the absence of tube formation (6).

In summary, LMWF, which is already known for its arterial antithrombotic properties in animals (7), induced a decrease in PAI-1 release by HUVEC associated with vascular tube formation. This effect could be beneficial for revascularization of ischemic areas.

References

1. Ribatti D, Urbinati C, Nico B, Rusnati M, Roncali L, Presta M. Endogenous basic fibroblast growth factor is implicated in the vascularization of the chick embryo chorioallantoic membrane. Dev Biol 1995; 170: 39-49.

2. Schnaper HW, Barnathan ES, Mazar A, Maheshwari S, Ellis S, Cortez SL, Baricos WH, Kleinman HK. Plasminogen activators augment endothelial cell organization in vitro by two distinct pathways. J Cell Physiol 1995; 165: 107-18.

3. DeLisser HM, Christofidou-Solomidou M, Strieter RM, Burdick MD, Robinson CS, Wexler RS, Kerr JS, Garlanda C, Merwin JR, Madri JA and Albelda SM. Involvement of endothelial PECAM-1/CD31 in angiogenesis. Am J Pathol 1997; 151: 671-77.

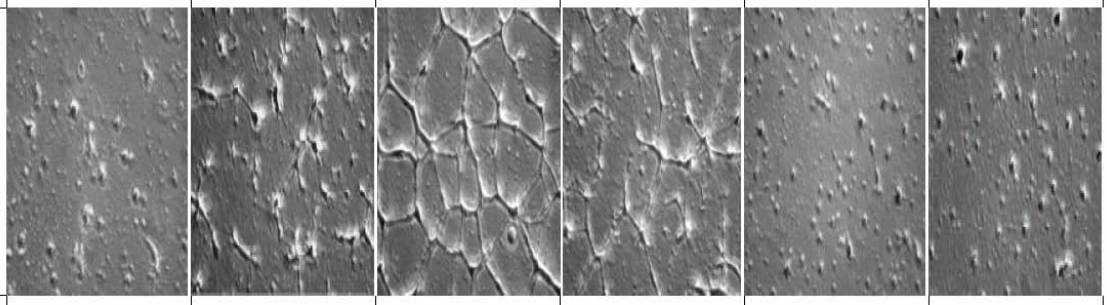
4. Griffioen AW, Damen CA, Martinotti S, Blijham GH, Groenewegen G. Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. Cancer Res 1996; 56: 1111-17.

5. Chabut D, Fischer AM, Colliec-Jouault S, Laurendeau I, Matou S, Le Bonniec B, Helley D. Low molecular weight fucoidan and heparin enhance the FGF-2-induced tube formation of endothelial cells through heparan sulfate-dependent alpha 6 over-expression. Mol Pharmacol (in press).

6. Xin X, Yang S, Kowalski J, Gerritsen ME. Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis in vitro and in vivo. J Biol Chem 1999; 274: 9116-21.

7. Colliec-Jouault S, Millet J, Helley D, Sinquin C and Fischer AM. Effect of low-molecular-weight fucoidan on experimental arterial thrombosis in the rabbit and rat. J of Thromb Haemost 2003; 1: 1114-15.

Figure 1 : HUVEC were grown for 72 h in medium supplemented with 5% FCS alone (untreated cells) or containing 10 µg/ml LMWF or dalteparin with or without 5 ng/ml FGF-2. The photos showing vascular tube formation on Matrigel are representative of a minimum of three experiments. Flow cytometry analysis for surface labeling of uPAR and ICAM-1 is expressed as a percent of the mean fluorescence intensity (mean ± SE of five experiments) of untreated HUVEC. ELISA assays for antigen levels of PAI-1 in cell supernatants are expressed as a percent of the PAI-1 release of untreated HUVEC.

	Untreated cells	FGF-2	FGF-2+ LMWF	FGF-2+ LMWH	LMWF	LMWH
Tube formation						
ICAM-1 labelling (% of control)	100%	Not expressed	Not expressed	Not expressed	174% +/- 12	187% +/- 14
uPAR labelling (% of control)	Not expressed	100%	98% +/- 9.12	107% +/- 4.9	Not expressed	Not expressed
PAI-1 release (% of control)	100%	54% +/- 5	14% +/- 1.5	55% +/- 9	107% +/- 9	100% +/- 17