Effect of unfractionated heparin and a low molecular weight heparin (enoxaparin) on coagulant activity of cultured human endothelial cells

VICENTA MARTINEZ-SALES, VIRTUDES VILA, EDELMIRO RÉGANON, JAVIER GARCIA OMS, JUSTO AZNAR

Background and Objectives. Unfractionated heparin and low molecular weight heparins exert their anticoagulant effect by mobilizing tissue factor pathway inhibitor (TFPI) from the vascular endothelium into the blood circulation. We compared the influence of unfractionated heparin and enoxaparin on the anticoagulant function of cultured human endothelial cells.

Design and Methods. Monolayers of human umbilical vein endothelial cells were treated with 10 U/mL unfractionated heparin or enoxaparin for different periods of time (30 min-48h). Endothelial cell procoagulant activity was determined in the cell lysates by a chromogenic assay. Endothelial cell tissue factor (TF) and released TFPI and von Willebrand factor (vWF) were determined.

Results. In short periods of incubation (30 min-2h), both heparins reduced endothelial cell procoagulant activity, the inhibition produced by unfractionated heparin being greater than that induced by enoxaparin ($p<0.05$). However, no variations were observed in TFPI and vWF release. With long periods of incubation (24-48h), both unfractionated heparin and enoxaparin significantly increased TFPI release (control vs. unfractionated heparin, $p<0.05$-0.001; control vs. enoxaparin, $p<0.01$-0.001) and also reduced the release of vWF in the culture medium, though no variations in endothelial cell procoagulant activity or TF content were observed.

Interpretation and Conclusions. Our findings show that unfractionated heparin and enoxaparin exert different kinds of effects on endothelial cells. With short incubation periods, procoagulant endothelial cell capacity was reduced to a greater extent by unfractionated heparin, while after longer periods of incubation enoxapain increased the anticoagulant activity of the endothelial cells to a greater degree than did unfractionated heparin.

Key words: heparin, enoxaparin, endothelial cells, tissue factor pathway inhibitor.

Haematologica 2003; 88:694-699
http://www.haematologica.org/2003_06/694.htm

From the Research Center (VM-S, VV, ER), Department of Obstetrics and Gynecology (JGO) and Department of Clinical Pathology (JA), La Fe University Hospital, Valencia, Spain.

Correspondence: Dr Vicenta Martínez-Sales, Ph D, Centro de Investigación, La Fe University Hospital, Avda. Campanar, 21, 46009- Valencia, Spain. E-mail: martinez_vicsal@gva.es

Endothelial cells play a central role in the regulation of hemostasis by ensuring the cellular control of both procoagulant and anticoagulant mechanisms. The extrinsic coagulation cascade is initiated when tissue factor (TF) is exposed at a site of blood vessel injury.[1] TF is a cell membrane integral protein receptor for circulating factor VII (FVII), and the rapid interaction of this factor and its receptor promotes the conversion of zymogen FVII to an active serine protease, activated factor VIIa (FVIIa).[2,3] The TF-FVIIa complex in turn activates factors IX and X, which leads to thrombin generation.[1,3] Thrombin induces procoagulant changes in endothelial cell function, including the release of von Willebrand factor (vWF).[4] The control of the highly procoagulant activity of the TF-FVIIa complex occurs through feedback inhibition by tissue factor pathway inhibitor (TFPI), which is considered to be the principal physiologic inhibitor of the complex.[1,5] TFPI is a serine protease produced chiefly by the endothelial cells.[5] A major portion of intravascular TFPI is stored associated with endothelial cells under normal condition.[6] TFPI is present in the plasma in free and lipoprotein-associated forms.[5] The free form of TFPI is released from the endothelial surface into plasma as a result of the action of heparin,[7] and exerts a much stronger anticoagulant effect than does the lipoprotein-associated form.[8]

Heparin administration *in vivo* causes prompt mobilization of TFPI into the circulation,[9] which is thus thought to contribute substantially to the anticoagulant action of heparin.[10] Both unfractionated heparin and low molecular weight heparins exert their anticoagulant effect by accelerating the inhibitory action of antithrombin III against its target sereine protease clotting factors (thrombin, factors IXa, Xa, XIa and XIIa),[11] and by mobilizing TFPI from the vascular endothelium into the blood circulation.[9] The exact profile of these effects depends on the molecular weight of the heparin. Enoxaparin is a low molecular weight heparin indicated for use in the treatment of coronary ischemic complications of unstable angina and non-Q wave myocardial infarction. Unfractionated heparin has for many years represented the standard in anticoagulation therapy for patients with acute coronary syndromes; however, recent studies suggest that enoxaparin is also a viable option for anticoagulant therapy in these patients. Data from the ESSENCE[12] and TIMI 11B[13] studies reported twice daily enoxaparin to be significantly more effective than unfractionated heparin in contin-

uous infusion in reducing death and serious cardiac ischemic events. Compared to unfractionated heparin, enoxaparin has 6-fold less anti-FIIa action and half the anti-FXa activity. Consequently, the ratio of anti-FXa to anti-FIIa activity is more than 3 for enoxaparin, whereas it is 1 for unfractionated heparin.[14] *In vitro* studies[15] have examined the ability of unfractionated heparin to modulate the procoagulant activity of stimulated endothelial cells, showing that unfractionated heparin reduces the procoagulant properties of stimulated endothelial cells. A recently reported study showed,[16] in a spontaneously transformed immortal endothelial cell line (ECV304), that the procoagulant activity of the cells was downregulated by 36%, and the contribution of TFPI to the anticoagulant potency of ECV304 cells was moderately increased after 24 hours of heparin stimulation. It is suggested that TFPI release is of major importance for the anticoagulant function of heparins. The aim of the present study was to compare the influence of unfractionated heparin and enoxaparin on the anticoagulant function of cultured human endothelial cells.

Design and Methods

Human umbilical vein endothelial cell culture

Endothelial cells from human umbilical cord veins (HUVEC) were obtained by collagenase digestion and were grown to confluent monolayers according to a method previously described.[17] HUVEC were grown in T-25 flasks pre-coated with endothelial cell attachment factor (Sigma), in Medium 199 with 15 mM HEPES supplemented with 20% fetal calf serum, 1% endothelial cell growth factor (Sigma), 2 mM L-glutamine, 1 mM sodium pyruvate, 50 U/mL penicillin, and 50 μg/mL streptomycin sulfate in an atmosphere of 95% air – 5% CO_2. The confluent endothelial cell monolayers were harvested from the culture flasks with 0.25 % trypsin, 0.01% EDTA in 10 mM phosphate buffer, 150 mM NaCl, pH 7.4 (PBS), without Ca^{2+} and Mg^{2+}. Cells were plated in 24-well polystyrene culture plates, at a density of approximately 30×10^3 cells/well, precoated with endothelial cell attachment factor and grown to reach 80-100% confluence in the above-mentioned medium. Only cells from these first subcultures were exposed to the different experimental procedures.

Cell treatment with heparins

The endothelial cells were treated with unfractionated heparin (Rovi, 1000 U/mL) and low molecular weight heparin, enoxaparin (Decipar, Italfarmaco S.A. 10000 U/mL). Confluent monolayers of endothelial cells in 24-well plates were washed twice in Medium 199 with 15 mM HEPES (basal medium) and incubated with cultured medium (without antibiotics) suplemented with 2% fetal calf serum and unfractionated heparin or enoxaparin at 10 U/mL final concentration for 30 minutes, 2, 4, 8, 24 and 48 hours at 37°C. Controls without heparin were dosed in parallel dishes. At the end of the incubation periods with or without heparins, the supernatants were harvested, kept at –80°C until assay, and the cell monolayers were washed three times with PBS and processed.

Determination of endothelial cell procoagulant activity with a chromogenic assay

The procoagulant activity was measured in the cell lysates by a chromogenic assay using human plasma. In brief, cells were mechanically removed with rubber policemen and scraped off the plates with 0.5 mL of 0.9% NaCl, followed by transfer of the cell suspension to tubes. The cells were disrupted by 3 freeze-thaw cycles in liquid nitrogen. Endothelial cell procoagulant activity was determined by incubating 50 μL of HUVEC lysate with 50 μL of pooled normal plasma for 2 min at 37°C; then, 5 μL of 250 mM $CaCl_2$ were added and incubated for 20 min at 37°C. Thrombin generation was stopped by adding 10 μL of 200 mM EDTA. The amount of thrombin generated was determined using a thrombin chromogenic substrate (S-2238, Chomogenix-Instrumentration Laboratory Spa.). Absorbances at 405 nm were read and the corresponding amount of thrombin was determined using a standard curve with known dilutions of thrombin (Sigma). Procoagulant activity was defined as the amount of free thrombin generated by cells (mU thrombin/10^3 cells).

Tissue factor antigen assay

Endothelial cell TF content was determined on cell lysates using a commercial ELISA kit (Immubind Tissue Factor, American Diagnostica Inc.). Cell lysates were obtained by lyzing cells in Tris-buffer saline, pH 7.8 (TBS; 0.1 M Tris-HCl and 150 mM NaCl), containing 1% Triton X-100, 60 mM octyl-β-D-glucopyranoside and a cocktail of inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM aprotinin, and 10 mM EDTA)[18] for 30 minutes at 37°C with vigorous vortexing.

Released tissue factor pathway inhibitor assay

Antigenic concentrations of free TFPI in the supernatants of endothelial cells were determined using a commercial ELISA kit (Asserachrom free TFPI, Diagnostica Stago).

von Willebrand factor assay

vWF activity was determined in the supernatant of endothelial cells using a commercial ELISA kit (Asserachrom vWF, Diagnostica Stago).

Table 1. Endothelial cell procoagulant activity (mU thrombin/10^3 cell). Effect of unfractionated heparin or enoxaparin.

	Incubation time					
	30 min	*2 hours*	*4 hours*	*8 hours*	*24 hours*	*48 hours*
Control	670±45	645±55	595±50	575±80	620±120	610±40
UFH	185±55*	185±50 *	430±150	465±150	550±125	520±115
ENOX	215±50 *	350±80†‡	470±125	525±50	580±100	540±60

*Results are expressed in IU thrombin/mL as mean ± SD of 6 experiments done in duplicate. UFH, unfractionated heparin (10 U/mL); ENOX, enoxaparin (10 U/mL); Control, without heparin; *p< 0.001, heparin vs. control; †p< 0.01, heparin vs. control; ‡p< 0.05, UFH vs. ENOX.*

Table 2. Effect of unfractionated heparin or enoxaparin on HUVEC tissue factor (pg/10^3 cells).

	Incubation time			
	4 hours	*8 hours*	*24 hours*	*48 hours*
Control	4.41±0.41	4.54±0.29	4.34±0.36	4.02±0.34
UFH	4.34±0.34	4.51±0.39	3.90±0.32	4.07±0.61
ENOX	4.97±0.49	4.85±0.44	4.07±0.27	3.95±0.41

Results are expressed as mean ± SD of 6 experiments done in duplicate. UFH, unfractionated heparin (10 U/mL); ENOX, enoxaparin (10 U/mL); Control, without heparin.

Statistical analysis

Results were expressed as the mean ± standard deviation (SD) of six experiments performed in duplicate. An unpaired t-test for normal distributions was used for the statistical comparison between controls and heparin-treated cells. Statistical comparisons between groups at different time points were performed using a paired-samples t-test. The SPSS version 10.0 statistical package was used throughout. A p value of ≤ 0.05 was considered statistically significant.

Results

Endothelial cell procoagulant activity

The endothelial cell procoagulant activity levels in HUVEC without heparin, with unfractionated heparin (10 U/mL) or with enoxaparin (10 U/mL) incubated at 37°C for different periods (30 min, 2, 4, 8, 24, and 48 h) were measured.

The results show that the interaction of unfractionated heparin and enoxaparin with HUVEC significantly decreased endothelial cell procoagulant activity by 30 minutes and 2 hours of incubation compared to the control (Table 1). Both unfractionated heparin and enoxaparin reduced endothelial cell procoagulant activity to the same extent at 30 min (70%, $p<0.001$). However, at 2 hours of incubation, unfractionated heparin (70%, $p<0.001$) produced greater endothelial cell procoagulant activity inhibition than did enoxaparin (45%, $p<0.01$): the difference between the effects of the two heparins was statistically significant (Table 1). After four hours of treatment with either unfractionated heparin or enoxaparin the observed procoagulant activity of the endothelial cells was slightly but not significantly less than that in the control group experiments (Table 1). Endothelial cell procoagulant activity was not modified by 8 to 48 hours of incubation (Table 1).

Tissue factor

We determined the endothelial cell TF content in cell extracts of HUVEC incubated at 37°C for 4, 8, 24 and 48 hours without heparin or with unfractionated heparin (10 U/mL) or with enoxaparin (10 U/mL). The endothelial cell TF content under basal conditions and in heparin-conditioned media at different time points are shown in Table 2. No significant differences in endothelial cell TF content were found between the control cells and HUVEC treated with UFH or with enoxaparin at any time point (Table 2). No significant differences were observed in endothelial cell TF content in relation to the two study treatments.

Tissue factor pathway inhibitor

We determined the free TFPI in the cultured media of HUVEC incubated at 37°C for 30 minutes and 2, 4, 8, 24, and 48 hours without heparin, with unfractionated heparin (10 U/mL) or with enoxaparin (10 U/mL). Results of free TFPI release by HUVEC without heparin and in heparin-conditioned media at different times are shown in Table 3. Both unfractionated heparin and enoxaparin induced a time-dependent and significant increase in free TFPI secretion (Table 3). After short periods (30 min- 4 h) of incubating HUVEC with unfractionated heparin or with enoxaparin, no significant variations were observed in endothelial cell-released free TFPI (Table 3). After 8 hours of incubation, only enoxaparin increased free TFPI release by the endothelial cells - this increase being significant compared with the release from both the control cells and the cells treated with unfractionated heparin. At 24 and 48 hours of incubation with heparins, unfractionated heparin and enoxaparin both induced a time-dependent and significant increase in free TFPI release ($p<0.05$ and $p<0.001$, respectively) (Table 3). Comparing the effects of the two heparins, it was seen that free

Table 3. Free tissue factor pathway inhibitor released (ng/10⁶cells) by HUVEC. Effect of unfractionated heparin and enoxaparin.

	Incubation time					
	30 min	*2 hours*	*4 hours*	*8 hours*	*24 hours*	*48 hours*
Control	2.8±0.3	3.3±0.4	3.9±0.7	5.0±0.6	6.0±3.5	12.6±3.2
UFH	2.8±0.4	3.3±0.3	4.3±0.3	5.8±0.9	15.7±7.5 *	30.0±3.6†
ENOX	2.8±0.3	3.4±0.5	4.7±0.1	7.2±0.7‡§	19.9±10.4*§	39.8±7.7†°

*Results are expressed as mean ± SD of 6 experiments done in duplicate; UFH, unfractionated heparin (10 U/mL); ENOX, enoxaparin (10 U/mL); Control, without heparin; * p< 0.05, heparin vs. control; †p< 0.001, heparin vs. control; ‡p< 0.01, heparin vs. control; §p< 0.01, UFH vs. ENOX; °p< 0.001, UFH vs. ENOX*

Table 4. Effect of unfractionated heparin or enoxaparin on HUVEC release of von Willebrand factor (% activity/10⁶cells).

	Incubation time					
	30 min	*2 hours*	*4 hours*	*8 hours*	*24 hours*	*48 hours*
Control	4.9±0.4	4.7±0.2	3.9±0.3	6.1±0.1	5.8±0.7	22.8±0.8
UFH	4.6±0.3	4.2±0.5	4.4±0.4	5.8±0.3	5.6±0.5	15.2±1.8 *
ENOX	4.7±0.9	4.0±0.4	4.7±0.2	6.1±0.1	5.2±0.4	15.4±0.9†

*Results are expressed as mean ± SD of 6 experiments done in duplicate. UFH, unfractionated heparin (10 U/mL); ENOX, enoxaparin (10 U/mL); Control, without heparin; *p< 0.05, UFH vs. control; †p<0.01, ENOX vs. control.*

TFPI release was greater with enoxaparin than with unfractionated heparin after both 24 (p<0.01) and 48 hours (p<0.001).

von Willebrand factor

HUVEC-released vWF was measured as the percentage activity of vWF in the cell culture media. We determined the vWF in the cultured media of HUVEC incubated at 37°C for 30 minutes and 2, 4, 8, 24, and 48 hours without heparin, with unfractionated heparin (10 U/mL) or with enoxaparin (10 U/mL). Results of vWF release by HUVEC without heparin and in heparin-conditioned media at different time points are shown in Table 4. The results show that during the first 24 hours of incubation with heparins, vWF release changed as a result of the action of heparin (Table 4). After 48 hours of incubation with heparin (UFH or enoxaparin) there was a significant decrease in vWF release by HUVEC (p<0.05 and p<0.01, respectively) (Table 4). However, when the effects of unfractionated heparin and enoxaparin were compared, no significant differences were recorded between the two heparins at any time.

Discussion

The present study shows that the interaction of heparin with endothelial cells modulates the anticoagulant potential of endothelial cells. First, a short period of incubation of heparins with endothelial cells resulted in potent inhibition of the procoagulant activity of the cells. Both unfractionated heparin and enoxaparin reduced endothelial cell procoagulant activity - though the reduction was comparatively greater with unfractionated heparin than with enoxaparin. Heparins stimulate the synthesis and the accumulation of heparan sulfate from endothelial cells[19,20] and this endothelial heparan sulfate shows anticoagulant activity.[21] It seems reasonable that the reduced thrombin generation exhibited by endothelial cells when they are exposed to heparins could be due, at least in part, to increased production of heparan sulfate by the endothelial cells. However, no differences in endothelial cell antigenic TF were found between the control cells and cells treated with unfractionated heparin or enoxaparin at any point of incubation. These results are in accordance with those of Cadroy *et al.*[15] who found that unfractionated heparin reduced coagulation activity, TF activity and prothrombinase activity, but did not modify TF antigen content in stimulated endothelial cells. Recently, other authors[22] reported that shear stress reduces functional but not antigenic expression of TF by intact activated endothelial cell monolayers.

In confirmation of previous findings, cellular release of free TFPI increases during prolonged incubation of endothelial cells with heparin, probably through up-regulation of TFPI expression and synthesis.[23] TFPI is produced predominantly by the endothelium, where it remains bound - presumably via glycosaminoglycan structures.[24] TFPI associated with the cell surface of the endothelial cells is thought to act as a direct vessel-wall anticoagulant. During the two first hours of endothelial cell treatment with either unfractionated heparin or enoxaparin we found a decrease in endothelial cell procoagulant activity, unrelated to TFPI release. This fact suggests that there might be some mechanism of inhibiting endothelial cell procoagulant activity that is not related to TFPI release. Accordingly, both heparins showed a biphasic action on endothelial cells: first endothelial cell procoagulant activity decreased, then this was followed by an increase in TFPI release. Comparisons between the effects of unfractionated heparin and enoxaparin showed that the latter induced greater free TFPI release by endothelial cells. Thus, enoxaparin is clearly more efficient than unfractionated heparin in increasing the functional activity of TFPI in endothelial cells *in vitro*. Recently, Alban *et al.*[25] performed a clinical study in healthy volunteers and found that unfractionated heparin mobilized

more free, but not total, TFPI than did enoxaparin. The apparent differences between *in vivo* and *in vitro* conditions could be related to the lower lipoprotein concentration in culture medium than in blood. *In vivo* unfractionated heparin efficiently prevents the binding of plasma lipoproteins to the released TFPI, whereas shorter heparin molecules with lower affinity to TFPI are partly displaced by lipoproteins.[25]

Our *in vitro* data could be interesting in relation to recent publications describing that the use of enoxaparin in patients receiving fibrinolytic therapy for acute myocardial infarction is associated with fewer acute cardiac events than is the use of unfractionated heparin,[26] and that enoxaparin treatment reduces both cerebral lesions and functional defects induced by local ischemia.[27] Our results show that both forms of heparin produce less release of vWF. Better control of vWF plasma levels means less FVIIIa is available for thrombin generation, which is a major agonist of both endothelial cells and platelets. Less thrombin generation may further limit the release of vWF stored in Weibel-Palade and α-granules,[28] and previous reports have shown that therapeutic doses of enoxaparin do significantly reduce thrombin generation.[20,30]

In conclusion, the present study shows that unfractionated heparin and enoxaparin exert two different kinds of effects on the procoagulant activity of endothelial cells. With short incubation times, both heparins reduce plasma thrombin generation, acting as a direct vessel-wall anticoagulant, with effect of unfractionated heparin being greater. After long periods of incubation, however, enoxaparin increases the anticoagulant activity of the endothelial cells to a greater extent than does unfractionated heparin.

References

1. Broze GJ Jr, Warren LA, Novotny WF. Regulation of coagulation by a multivalent Kunitz-type inhibitor. Biochemistry 1990;29:7530-46.
2. Edgington TS, Mackman N, Brand K, Ruf W. The structural biology of expression and function of tissue factor. Thromb Haemost 1991;66:67-79.
3. Nemerson Y. Tissue factor: then and now. Thromb Haemost 1995;74:180-4.
4. Levine JD, Harlan JM, Harker LA, Joseph ML, Counts RB. Thrombin-mediated release of factor VIII antigen from human vein endothelial cell in culture. Blood 1982;60:531-4.
5. Bajaj MS, Birktoft JJ, Steer SA, Bajaj SP. Structure and biology of tissue factor pathway inhibitor. Thromb Haemost 2000;86:959-72.
6. Lupu C, Lupu F, Dennehy U, Kakkar VV, Scully M. Thrombin induces the redistribution and acute release of tissue factor pathway inhibitor from specific granules within human endothelial cells in culture. Arterioscler Thromb Vasc Biol 1995;15:2055-62.
7. Hansen JB, Huseby KR, Huseby NE, Sandset PM, Hanssen TA, Nordoy A. Effect of cholesterol lowering on intravascular pools of TFPI and its anticoagulant potential in type II hyperlipoproteinemia. Arterioscl Throm Vas Biol 1995;15:879-85.
8. Lindahl AK, Sandset PM, Abildgaard U. The present status of tissue factor pathway inhibitor. Blood Coagul Fibrinolysys 1992;3:439-49.
9. Sandset PM, Abildgaard U, Larsen ML. Heparin induces release of extrinsic coagulation pathway inhibitor (EPI). Thromb Res 1988;50:803-13.
10. Lindahl AK, Abildgaard U, Staalesen R. The anticoagulant effect in heparinized blood and plasma resulting from interactions with extrinsic pathway inhibitor. Thromb Res 1991; 64: 155-68.
11. Barrowcliffe TW. Low molecular weight heparin(s). Br J Haematol 1995;90:1-7.
12. Cohen M, Demers C, Gurfinkel EP, Turpie AG, Fromell GJ, Goodman S, et al. A comparison of low-molecular-weight heparin with unfractionated heparin for unstable coronary artery disease. Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q-Wave Coronary Events Study Group. N Engl J Med 1997;337:447-52.
13. Antman EM, McCabe CH, Gurfinkel EP, Turpie AG, Bernink PJ, Salein D, et al. Enoxaparin prevents death and cardiac ischemic events in unstable angina/non-Q-wave myocardial infarction. Results of the thrombolysis in myocardial infarction (TIMI) 11B trial. Circulation 1999;100:1593-601.
14. Buckley MM, Sorkin EM. Enoxaparin. A review of its pharmacology and clinical applications in the prevention and treatment of thromboembolic disorders. Drugs 1992;44:465-97.
15. Cadroy Y, Gaspin D, Dupouy D, Lormeau JC, Boneu B, Sié P. Heparin reverses the procoagulant properties of stimulated endothelial cells. Thromb Haemost 1996;75:190-5.
16. Hansen JB, Svensson B, Olsen R, Ezban M, Osteud B. Heparin induces synthesis and secretion of tissue factor pathway inhibitor from endothelial cells in vitro. Throm Haemost 2000; 83:937-43.
17. Martínez-Sales V, Gómez-Lechón MJ, Gilabert J. Characteristics of arachidonic acid metabolism of human endothelial cells in culture. Cytotechnology 1990;3:21-9.
18. Westmuckett AD, Lupu C, Roquefeuil S, Krausz T, Kakkar VV, Lupu F. Fluid flow induces upregulation of synthesis and release of tissue factor pathway inhibition in vitro. Arterioscler Thromb Vasc Biol. 2000;20:2474-82.
19. Nader HB, Buonassisi V, Colburn P, Dietrich CP. Heparin stimulates the synthesis and modifies the sulfation of heparin sulfate proteoglycan from endothelial cells. J Cell Phisiol 1989;140:305-10.
20. Pinhal MAS, Santos IAN, Silva IF, Dietrich CP, Nader HB. Minimum fragments of heparin molecule are able to produce the accumulation and change of the sulfation pattern of an antithrombotic heparan sulfate from endothelial cells. Thromb Haemostasis 1995;74:1169-74.
21. Mertens G, Cassiman JJ, Van der Berghe H, Vermylen J, David G. Cell surface heparan sulfate proteoglycans from vascular endolthelial cells. Core protein characterization and antithrombin III binding properties. J Biol Chem 1992;267: 20435-43.
22. Grabowski EF, Reininger AJ, Petteruti PG, Tsukurov O, Orkin RW. Shear stress decreases endothelial cell tissue factor activity by augmenting secretion of tissue factor pathway inhibitor. Arterioscler Thromb Vasc Biol 2001;21:157-62.
23. Lupu C, Poulsen E, Roquefeuil S, Westmuckett AD, Kakkar VV, Lupu F. Cellular effects of heparin on the production and release of tissue factor pathway inhibitor in human endothelial cells in culture. Arterioscler Thromb Vasc Biol 1999;19:2251-62.
24. Novorty WF, Brown SG, Miletich JP, Rader DJ, Broze GI. Plasma antigen levels of the lipoprotein-associated coagulation inhibitor in patients samples. Blood 1991;78:387-93.
25. Alban S. Molecular weight-dependent influence of heparin on the form of tissue factor pathway inhibitor circulating in plasma. Semin Thromb Hemost 2001;27:503-11.
26. Baird SH, Menown IBA, McBride SJ, Trouton TG, Wilson C. Randomized unfractionated heparin following fibrinolytic therapy for acute myocardial infaction. Eur Heart J

2002;23:627-32.
27. Mary V, Wahl F, Uzan A, Stutzmann JM. Enoxaparin in experimental stroke. Neuroprotection and therapeutic window of opportunity. Stroke 2001;32:993-9.
28. Vischer UM, Ingerslev J, Wollheim CB, Mestries JC, Tsakiris DA, Haefell WE, et al. Acute von Willebrand factor secretion from the endothelium in vivo: assessment through plasma propeptide (vWf:AgII) levels. Thromb Haemost 1997;77:387-93.
29. Bara L, Bloch MF, Zitoun D, Samama M, Collignon F, Frydman A, et al. Comparative effects of enoxaparin and unfractionated heparin in healthy volunteers on prothrombin and consumption in whole blood during coagulation, and release of tissue factor pathway inhibitor. Thromb Res 1993;69:443-52.
30. Beguin S, Mardiguin J, Lindhout T, Hemker HC. The mode of action of low molecular weight heparin preparation (PK10169) and two of its major components on thrombin generation in plasma. Thromb Haemost 1989;61:30-4.

Pre-publication Report & Outcomes of Peer Review

Contributions

VMS, VV, ER contributed to the conception and design of the study, they carried out part of the analytical assay and contributed to the analysis and interpretation of the results. JGO was involved in obtaining the umbilical cords and contributed to data analysis and interpretation. JA critically revised the different versions of the manuscript. The order in which the names appear is based on the time spent by each contributor on this research. The authors wish to thank Guadalupe Manzano, Aurelia Royo, Ursula Salinas and Natalia García-Esteve for their expert technical assistance. Primary responsibility for the publication and for each Table and Figure: VMS.

Funding

This work was supported in part by Grant 00/1063 from the Fondo the Investigaciones Sanitarias (FIS) del Ministerio de Sanidad y Consumo, Spain.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received October 10, 2002; accepted March 7, 2003.

In the following paragraphs, Professor Cazzola summarizes the peer-review process and its outcomes.

What is already known on this topic

Unfractionated heparin and low molecular weight heparins exert their anticoagulant effect by mobilizing tissue factor pathway inhibitor.

What this study adds

While unfractionated heparin is more efficient than low molecular weight heparin (enoxaparin) in reducing endothelial cell procoagulant activity, enoxaparin is more efficient in increasing the release of tissue factor pathway inhibitor, a crucial step in the anticoagulant function of heparins.