Low Molecular Weight Heparin Enhances Prostacyclin Production by Cultured Human Endothelial Cells

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Confluent cultures of vascular endothelial cells derived from the human umbilical vein were incubated in a serum-free medium in the presence of low molecular weight heparin (LMWH) with molecular weights of 4000–6000 dalton (Da), or of unfractionated heparin (UFH) with average molecular weight 12000 Da, and prostacyclin production was determined by radioimmunoassay for 6-keto-prostaglandin $F_1\alpha$, the stable metabolite of prostacyclin. LMWH at 1 U/ml as anti-factor Xa activity significantly increased prostacyclin production after 6 h or longer; however, UFH at 1 USP U/ml did not induce such a significant change. The LMWH-induced increase in prostacyclin production occurred at 0.1 U/ml and above after 6 h of treatment. Since prostacyclin is both a potent inhibitor of platelet aggregation and a vasodilator, it was suggested that the increased endothelial cell prostacyclin production induced by LMWH may be a component of the anticoagulant activity of the drug.

Keywords endothelial cell; heparin; low molecular weight heparin; prostacyclin; prostaglandin

Heparin is an anticoagulant that has been used clinically for almost 50 years. The anticoagulant activity of heparin depends on its interaction with antithrombin III, an inhibitor of thrombin and most other enzymes in the clotting cascade. In particular, antithrombin III inactivates the coagulation factor Xa as well as thrombin. Heparin induces a configurational change of antithrombin III, resulting in an approximate 1000-fold increase in its activity in neutralizing the clotting enzymes.¹⁾

The commercial preparations of heparin are heterogeneous and contain heparin molecules with molecular weights from 5000 to 30000 dalton (Da).²⁾ Recently, low molecular weight heparin (LMWH) with molecular weights of 3000 to 6000 has been attractive clinically, since its use is associated with less bleeding³⁾ which is the major side effect of unfractionated heparin (UFH). Also, there are some differences between UFH and LMWH on the activity of the coagulation factors.^{4,5)}

On the other hand, vascular endothelial cells have an anticoagulant activity which is mediated by the production of either prostacyclin,⁶⁾ anticoagulant heparan sulfate,⁷⁾ thrombomodulin⁸⁾ or tissue plasminogen activator.⁹⁾ Both UFH and LMWH enhance thrombin-stimulated prostacyclin production by cultured endothelial cells¹⁰⁾; UFH but not LMWH stimulates the production¹¹⁾ and release¹²⁾ of heparan sulfate from the cells; UFH increases tissue plasminogen activator production by cultured microvascular endothelial cells.¹³⁾

The present study was undertaken to clarify whether LMWH is individually capable of inducing prostacyclin production by cultured endothelial cells.

Materials and Methods

Materials Dulbecco's modified Eagle's medium and ASF 301 medium were purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan) and Ajinomoto Co., Ltd. (Tokyo, Japan), respectively. Fetal bovine serum (FBS) was from Bioproducts, Inc. (Walkersville, MD, U.S.A.). Endothelial cell growth supplement (ECGS) and UFH (average molecular weight 12000 Da) were obtained from Sigma Chemical Co. (St. Louis, MD, U.S.A.). LMWH (molecular weights 4000–6000 Da) was from KabiVitrum AB (Stockholm, Sweden). Tissue culture plates coated with type I collagen were purchased from Sumitomo Bakelite, Co., Ltd. (Tokyo, Japan). Vascular endothelial cells from the human umbilical vein were from Kurabo (Osaka, Japan). The 6-keto-prostaglandin $F_1\alpha$ (6-keto-PGF₁ α) [¹²⁵I]radioimmunoassay kit was obtained from

Amersham Corp. (U.K.).

Cell Culture Endothelial cells were cultured in 24-well culture plates with Dulbecco's modified Eagle's medium supplemented with 10% FBS and 200 μ g/ml ECGS at 37°C in a humidified chamber with an atmosphere of 5% CO₂ in air until confluent. The medium was then discarded and the cell layer was washed twice with serum-free ASF 301 medium was harvested and the content of 6-keto-PGF₁ α , the stable metabolite of prostacyclin, was measured by radioimmunoassay. The activity of UFH and LMWH were expressed as USP units and anti-factor Xa activity, respectively; however, the amount (μ g) of UFH at 1 USP unit was almost equal to that at 1 anti-factor Xa activity.

Statistical Analysis The effect of LMWH was assessed by analysis of variance. *p* values less than 0.05 were considered to indicate a statistically significant difference.

Results

Since the blood concentration of heparin reaches about 1 U/ml in cases where the drug is used clinically, we

TABLE I. Comparison of the Effect of LMWH and UFH on the Production of Prostacyclin by Cultured Endothelial Cells

	6-Keto-PGF ₁ α (pg/well)		
	2 h	6 h	8 h
Control	4.21 $\pm$ 0.97	3.99 $\pm$ 0.79	11.19 $\pm$ 1.56
LMWH	3.86 $\pm$ 1.09	6.82 $\pm$ 0.16	16.22 $\pm$ 0.79
UFH	4.97 $\pm$ 0.84	4.00 $\pm$ 0.97	9.72 $\pm$ 0.59

Confluent cultures of endothelial cells were treated with 1 U/ml LMWH or UFH for 2 or 6 or 8 h. Values are means $\pm$ S.E. of 4 samples. The effect of LMWH on the prostacyclin production was significant (*p* < 0.01).

TABLE II. Effect of LMWH on the Production of Prostacyclin by Cultured Endothelial Cells

	6-Keto-PGF ₁ α (pg/well)
Control	7.76 $\pm$ 0.76
0.1 U/ml LMWH	13.48 $\pm$ 1.51
1.0 U/ml LMWH	14.70 $\pm$ 0.47
10 U/ml LMWH	22.58 $\pm$ 2.54

Confluent cultures of endothelial cells were treated with 0.1 or 1.0 or 10 U/ml LMWH for 6 h. Values are means $\pm$ S.E. of 6 samples. The effect of LMWH on the prostacyclin production was significant (*p* < 0.01).

investigated the effect of 1.0 U/ml LMWH and UFH on the production of prostacyclin by cultured endothelial cells (Table I). LMWH significantly increased the prostacyclin production after 6 h. In the 6 h incubation, the prostacyclin production was unexpectedly insufficient for unknown reasons; however, LMWH-stimulated prostacyclin production was observed. UFH did not show such a significant change in the tested conditions. As shown in Table II, the LMWH-induced increase in prostacyclin production occurred at 0.1 U/ml and above.

Discussion

It has been proposed that heparin is capable of interacting with blood vessels. Heparin binds to the endothelium¹⁴⁾ and may exhibit an anticoagulant action on the surface. It is likely that the anticoagulant action of heparin is due not only to the anti-serine protease actions but also to a stimulation of the anticoagulant functions of endothelial cells. In the present study, we found that LMWH individually stimulates the production of prostacyclin by cultured human endothelial cells (Table II). Since prostacyclin is a potent inhibitor of platelet aggregation, this LMWH effect may contribute to the anticoagulant action of the drug.

We showed that UFH at 1.0 U/ml did not increase prostacyclin production (Table I); however, in another experiment, we observed that UFH at 10 U/ml did stimulate prostacyclin production. Although this UFH effect may be dependent on the content of LMWH in UFH, a possibility that high molecular weight heparin also can induce endothelial cell prostacyclin production is not excluded. In our preliminary experiment, exogenous heparan sulfate, dermatan sulfate and hyaluronic acid were each capable of inducing prostacyclin production (data not shown). Thrombin¹⁰⁾ and antithrombin III¹⁵⁾ each stimulate endothelial cell prostacyclin production; however, heparan sulfate on the cell surface mediates only the stimulation by antithrombin III.¹⁶⁾ Endothelial cells may have receptors for glycosaminoglycans which mediate

prostacyclin production by the cells.

In conclusion, it was shown that LMWH enhances the production of prostacyclin by cultured endothelial cells. Although the potential activity of LMWH appears to be much lower than that of thrombin^{6,10)} (prostacyclin production at 1 NIH U/ml for 20 min is approximately 200 pg/well in our system) and antithrombin III,¹⁵⁾ this LMWH effect may contribute to its anticoagulant action. This stimulatory effect of LMWH is an appropriate one as an anticoagulant drug compared with UFH. The effect of heparin on endothelial cell functions must be investigated further.

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