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Effect of enoxaparin on high glucose-induced activation of endothelial cells

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Abstract

Clinical and experimental studies indicate that low-molecular-weight heparins reduce inflammation. To uncover the possible mechanisms involved, we investigated the effect of a low-molecular-weight heparin, enoxaparin, on high glucose-induced activation of endothelial cells. Bovine valvular endothelial cells and human endothelial cell line, EA hy926 were cultured in medium containing 5 mM (normal glucose) or 33 mM (high glucose) glucose. Postconfluency, the cells were exposed for 48 h to high glucose in the absence or presence of 16 μg/ml enoxaparin and tested for monocyte adhesion, expression of cell adhesion molecules, and translocation of nuclear transcription factor-κB (NF-κB), using adhesion assays, enzyme-linked immunosorbent assay (ELISA), and Western blotting. Statistical data revealed that treatment with enoxaparin resulted in a significant decrease in monocyte adhesion, expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, translocation of NF-κB, as well as in the level of intracellular reactive oxygen species. These results suggest that enoxaparin reduces the high glucose-induced activation of endothelial cells by inhibiting monocyte adhesion through a mechanism that involves cell adhesion molecules and NF-κB.
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Keywords: Enoxaparin; Endothelial cell; Adhesion; NF-κB (nuclear factor-κB)

1. Introduction

Clinical and experimental studies indicate that heparins (both unfractionated and low-molecular-weight) have an anti-inflammatory effect, possible as a result of their ability to bind various proteins (chemokines, growth factors, enzymes and adhesion molecules) and cells (vascular endothelium and leukocytes) involved in the inflammatory process (Lever and Page, 2002). Upon binding to cell adhesion receptors such as L and P selectin, CD11b/CD18 and platelet endothelial cell adhesion molecule (PECAM 1), heparins have the potential to interfere with each event involved in inflammatory cell recruitment, namely, rolling, triggering, adhesion, and transmigration. Investigations of the effects of heparins on endothelial leukocyte interaction have been focussed on the interaction of heparin with the leukocyte ligands required for adhesion to endothelial cells There are few studies on the direct effects of heparins on the expression by endothelial cells of the adhesion molecules involved in leukocyte binding. In a recent paper (Manduteanu et al., 2002), we evaluated the anti-inflammatory potential of enoxaparin, a low-molecular-weight heparin (4300 Da), investigating the direct effect of the drug on endothelial cell activation. We demonstrated that enoxaparin (clexane) inhibits monocyte adhesion to tumor necrosis factor (TNF-α) or lipopolysaccharide-activated endothelial cells by a mechanism involving cell adhesion molecules. Thus, the activation and expression of adhesion molecules are major determinants of monocyte adherence to endothelial cells. In patients with type 2 diabetes, elevated plasma levels of endothelial cell adhesion molecules (Kado et al., 2001; Kim et al., 1994; Lampeter et al., 1992; Steiner et al., 1994) and enhanced monocyte adhesion to endothelium (Carantoni et al., 1997; Desfaits et al., 1998) have been documented. Also, experimental studies (on animals and cells in culture) demonstrated that under high glucose conditions (a situation simulating the diabetic milieu), an

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increase in leukocyte adhesion to endothelium and an enhanced expression of endothelial cell adhesion molecules occur (Kado and Nagata, 1999; Manduteanu et al., 1999; Morigi et al., 1998; Renier et al., 2000). Since we have previously shown that high glucose enhances the expression of adhesion molecules and the ensuing monocyte adhesion to heart valve endothelial cells, and that enoxaparin reduces both these processes in TNF-α- or lipopolysaccharide-activated endothelium, we questioned whether the drug also has an effect on monocyte adhesion to high glucose-activated endothelial cells, a condition often occurring in diabetes. As a model system, we used either cultured endothelial cells either derived from cardiac valves valvular endothelial cells, which we have shown to be more reactive to glucose than endothelial cells from other locations (Manduteanu et al., 1998), or cells of the human endothelial cell line EAhy926, a line derived from human umbilical vein endothelial cells (Edgell et al., 1983). We report here that enoxaparin reduces high glucose-induced activation of valvular endothelial cells and human endothelial cells by repressing the expression of endothelial cell adhesion molecules and monocyte adhesion through a process involving the nuclear transcription factor nuclear factor-κB (NF-κB).

2. Materials and methods

2.1. Cells

Valvular endothelial cells were cultured as described (Manduteanu et al., 1988) and grown in normal, 5 mM, or high, 33 mM, glucose concentrations in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal calf serum. Cells at confluence were used between passages 3 and 5.

Human endothelial cells. The EAhy926 cell line (derived from human umbilical vein endothelial cells) was kindly donated by Dr. Cora Jean Edgell (Department of Pathology, University of North Carolina, Chapel Hill). The cells were grown in Petri dishes (60-mm diameter) or in tissue culture plates of 6, 24 or 96 wells.

U937 cells. These monocyte-like-cells (a kind gift from Prof. S.C. Silverstein from Columbia University, New York, USA) were grown in suspension in RPMI 1640 culture medium containing 5% fetal calf serum and were divided (1:5) twice a week.

2.2. Reagents

Enoxaparin (Clexane) was purchased from Aventis. The dye 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) was obtained from Molecular Probes (Eugene, OR, USA). The monoclonal antibodies to endothelial cell adhesion molecules intercellular adhesion molecule-1 (ICAM-1) mouse IgG_1 clone # BBIG-I1 (11C81), E-selectin mouse IgG_1 clone # BBIG-E4 (5D11), vascular cell adhesion molecule-1 (VCAM-1)) mouse IgG_1 clone # BBIG-V1 (4B2), and mouse immunoglobulin (IgG1) (isotope control) were purchased from R&D Systems, UK. The monoclonal antibody to p65 subunit of the transcription factor NF-κB was purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA. The kit for detection of p65 antibody was the Super Signal West Pico Chemiluminiscent Substrate and was purchased from Pierce (Rockford, USA). The fluorescein-labeled goat anti-mouse antibody (FITC-IgG) and all the other reagents were obtained from Sigma-Aldrich Chemie (Germany).

2.3. Test for monocyte adhesion to high glucose-activated endothelial cells

Confluent cultured valvular endothelial cells or human endothelial cells were subjected to two experimental conditions: (i) cells were grown in the presence of 5 mM (normal) glucose or (ii) cells were grown in 33 mM (high) glucose and further exposed for 48 h postconfluency to high glucose in the absence or presence of enoxaparin (16 μg/ml). Subsequently, the endothelial cells were washed with warm culture medium and then incubated with U937 cells (500,000/ml) for 30 min at 37 °C, in RPMI 1640 culture medium supplemented with 1% fetal calf serum. After being washed with the same culture medium (3×1 min) to remove the nonadherent monocytes, the monolayers were fixed with 2% paraformaldehyde in 0.1 mol/l phosphate buffer for 20 min, washed with phosphate-buffered saline (PBS), and examined by phase contrast microscopy. The adherent monocytes were counted on five microscopic fields per sample (in duplicate for each experimental condition). The data obtained are expressed as the number of bound monocytes per microscopic field. Statistical processing was performed using the One-Way ANalysis Of VAriance between groups (ANOVA) Program of Origin.

2.4. Evaluation of the effect of enoxaparin on cell adhesion molecules induced by high glucose on endothelial cells by enzyme-linked immunosorbent assay (ELISA)

Normal or activated valvular endothelial cells were rinsed twice with the culture medium and lightly fixed with 1% paraformaldehyde in 0.1 M phosphate buffer at room temperature. After 20 min, the fixative was replaced by PBS supplemented with 1% bovine serum albumin (PBS-A) buffer, and the cells were sequentially incubated for 1 h with PBS-containing 3% albumin (a blocking buffer) and for 2 h with the monoclonal antibodies to ICAM-1, E-selectin or VCAM-1 (10 μg/ml). After being washed, the cells were incubated with horseradish peroxidase conjugated mouse IgG (1:1000) for 1 h at room temperature and then washed again with PBS-A buffer. Then, 2 mg/ml *O*-phenylenediamine, 0.003% H_2O_2 in 0.1 M citrate-phosphate buffer, pH 4 (the substrate for the peroxidase reaction), was added and after 15 min, the reaction was stopped with 2 M sulfuric

acid; the absorbance at 492 nm was determined using an ELISA plate reader (Multiscan) as described by Hashemi et al. (1987).

2.5. Western blot analysis

To study if enoxaparin has an effect on the activation of transcription factor NF-κB, human endothelial cells were grown in DMEM containing (i) a normal glucose concentration; (ii) a high glucose concentration and (iii) a high glucose concentration followed by exposure for 48 h to enoxaparin. After that, all cells were subjected to Western blot analysis. For this purpose, human endothelial cells were lysed in a lysis buffer containing 10 mM HEPES pH 7.6, 15 mM KCL, 2 mM $MgCl_2$, 0.1 mM dithiothreitol, 1% Triton X-100, 0.5 mM phenylmethyl sulfonylfluoride and 5 μg/ml of inhibitors (benzamidine, pepstatin, and leupeptin). The nuclear and cytosolic fractions were separated by centrifugation ($650 \times g$, 10 min) and the protein concentrations of the fractions were determined by Amido black assay. Proteins (80 μg) were separated on a 12% polyacrylamide gel, transferred to nitrocellulose membrane Immunobilon polyvinylidene difluoride (PVDF), blocked 2 h with buffer containing 5% nonfat dry milk, and incubated overnight with 2 μg/ml of anti-p65 subunit of NF-κB (primary antibody). After being washed four times with buffer, the membranes were incubated with the second antibody (diluted 1:10,000) followed by enhanced chemiluminiscence kit (ECL) for 5 min and exposed to an X-ray film. The films were analyzed with a video system (Image Master from Pharmacia) and the optical density of the bands was calculated with the Total Lab 1.11 software from Pharmacia.

2.6. Determination of intracellular reactive oxygen species

Intracellular accumulation of reactive oxygen species was monitored by fluorimetry using 2′,7′-dichlorofluorescein diacetate (DCFH-DA) as reported earlier (Hempel et al., 1999; Zhang et al., 2000). The nonfluorescent dye DCF-DA is freely permeable into endothelial cells; inside the cells, the dye is hydrolyzed to 2′,7′-dichlorofluorescein (DCF), where upon interaction with reactive oxygen species, it is converted into a fluorescent DCF. Briefly, the cells were grown in normal or high glucose in six-well tissue culture plates and postconfluency, they were incubated with high glucose-containing medium in the absence or presence of 16 μg/ml enoxaparin. After 48 h, the cells were trypsinized, washed and loaded with 10 μmol/l DCFH-DA in Hanks' balanced salt solution (HBSS) for 45 min at 37 °C. After being washed twice in HBSS buffer, cell fluorescence was measured with a Shimadzu model RF 5001 PC fluorescence spectrophotometer at an emission wavelength of 535 nm and an excitation wavelength of 505 nm. The level of reactive oxygen species is expressed as mean fluorescence units per milligram of protein ± S.E.

2.7. Statistical analysis of the data

Statistical analysis was performed using the ANOVA program of Origin and a Student's *t*-test for a single comparison. Statistical significance was considered as P value < 0.05.

3. Results

3.1. Effect of enoxaparin on monocyte adhesion to high glucose-activated endothelial cells

Valvular endothelial cells and human endothelial cells grown in the presence of a normal or high glucose concentration with or without enoxaparin were exposed to monocytes in the absence or presence of enoxaparin. Valvular endothelial cells growing in a high glucose concentration (33 mM) had an about fourfold increased adhesion of monocytes as compared to controls, i.e. cells grown in normal glucose (Fig. 1A). Monocyte adhesion to human endothelial cells cultured in high glucose increased by about threefold compared to controls (Fig. 1B). Since the monocyte adhesion tests for valvular endothelial cells and human endothelial cells showed that growing the cells in the presence of a high glucose concentration resulted in a more adhesive surface on valvular endothelial cells than on human endothelial cells, we can assume that endothelial cells originating from the heart valves have an increased propensity to be activated by high glucose concentrations, a result that corroborates our previous data.

Further, we tested the effects of the low-molecular-weight heparin, enoxaparin, on the high glucose-induced

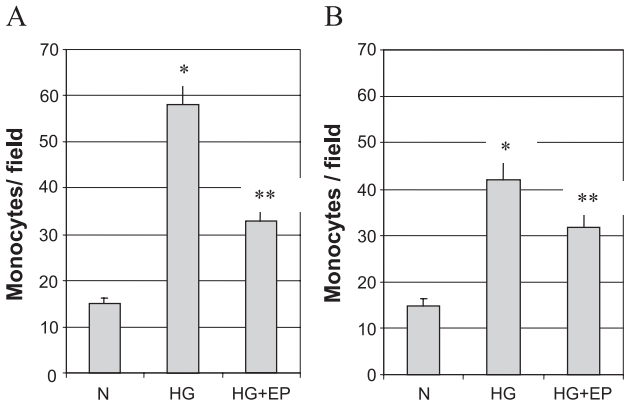

Fig. 1. Adhesion of monocytes to valvular endothelial cells (VEC) (A) and human endothelial cells (HEC) (B) grown in the presence of a high glucose concentration and the effect of enoxaparin on monocyte adhesion. The cells were grown under normal (N) and high glucose (HG) conditions, and upon attaining confluence, the adhesion test was applied in the absence or presence of enoxaparin (EP). Note that as compared to N conditions, monocyte adhesion was significantly increased in VEC and HEC cultured under HG condition (*significantly different from control, $P < 0.05$) and that EP reduced adhesion (**significantly different from HG, $P < 0.05$). Data are expressed as means ± S.E. for three independent experiments.

endothelial monocyte adhesion. The adhesion assay, performed after treatment of endothelial cells for 48 h with 16 μg/ml enoxaparin, revealed that the number of monocytes adherent to cells grown in high glucose was decreased significantly, namely by about 43% in the case of valvular endothelial cells (Fig. 1A) and by about 23% in the case of human endothelial cells (Fig. 1B). These data indicated that the drug is more effective in inhibiting the adhesion process in valvular endothelial cells than in human endothelial cells.

3.2. Searching for the molecular mechanisms involved in the effect of enoxaparin on monocyte adhesion to endothelial cells

3.2.1. Effects of enoxaparin on the expression of endothelial cell adhesion molecules induced by high glucose

To elucidate the effect of enoxaparin on the expression of adhesion molecules by endothelial cells grown under high glucose conditions, the cells (valvular endothelial cells or human endothelial cells) were grown to confluence in the presence of a normal or high glucose concentration, and then exposed for 48 h to high glucose in the absence or presence of enoxaparin.

ELISA assay revealed that 7 days after the endothelial cells were exposed to 33 mM glucose, the cells exhibited an enhanced expression of the endothelial cell adhesion molecules ICAM-1, VCAM-1 and E-selectin. As shown in Fig. 2A, in the case of valvular endothelial cells, the percentage over the control values was ~ 168 ± 8% for ICAM-1, ~ 229 ± 10% for VCAM-1, and ~ 162 ± 10% for E-selectin (Fig. 2A). Also, human endothelial cells cultivated under high glucose conditions exhibited an enhanced expression of cell adhesion molecules, amounting to 174 ± 8% for ICAM-1, to 138 ± 10% for VCAM-1 and to 186 ± 8% for E-selectin, over the control values (Fig. 2B). These results revealed that a high glucose concentration produced similar increases in ICAM-1 and E-selectin expression in both cell types compared to basal conditions. Nonetheless, in response to high glucose conditions, it was found that VCAM-1 surface expression increased more on valvular endothelial cells (2.29-fold increase over control) than on human endothelial cells (1.4-fold increase over control value), indicating that VCAM-1 may be instrumental to the higher adhesion of monocytes to valvular endothelial cells than to human endothelial cells under high glucose conditions. Exposure of cells to a high glucose concentration in the presence of enoxaparin showed that the drug reduced to a different degree, but significantly, the expression of adhesion molecules on valvular endothelial cells. Thus, the expression of ICAM-1 was reduced by 10%, that of E-selectin by 21% and that of VCAM-1 by 23% (Fig. 2A). As shown in Fig. 2B, in similar experiments, treatment with enoxaparin of human endothelial cells grown under high glucose conditions reduced significantly, but to a comparable degree, the expression of ICAM-1 (11%), E-selectin (10%) and VCAM-1 (10%). The data indicated that

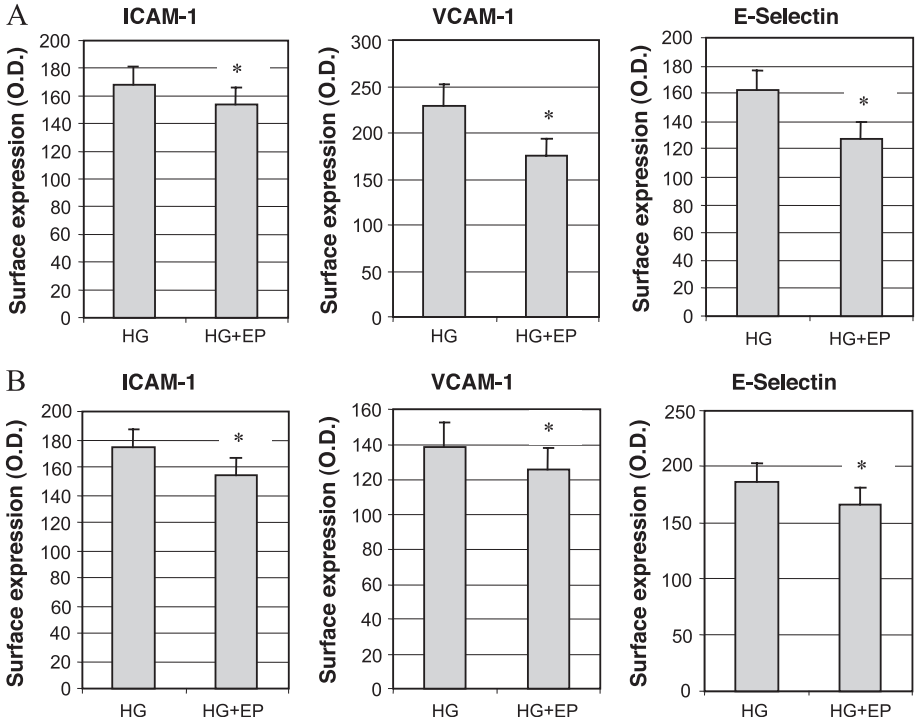

Fig. 2. Inhibitory effect of enoxaparin on high glucose (HG)-induced endothelial cell adhesion molecule expression on VEC (panel A) and HEC (panel B). The cells grown in the presence of HG were incubated after confluency for 48 h in the presence of HG or in HG plus enoxaparin (HG + EP). Note the significant effect of enoxaparin on the expression of cell adhesion molecules. Results are expressed as % over control values considered 100%. Data represent the means ± S.E. of three different experiments. *$P < 0.05$.

the drug has similar effects on the surface expression of ICAM-1 in valvular endothelial cells and human endothelial cells, whereas it is more potent in the attenuation of the expression of E-selectin and VCAM-1 in valvular endothelial cells.

3.2.2. Effect of enoxaparin on the activation of NF-κB

Since the experiments showed that enoxaparin is able to reduce the expression of endothelial cell adhesion molecules induced by high glucose and knowing that, in other cells, their transcription is influenced by the activation of NF-κB, we performed experiments to evaluate whether the drug interferes with the activity of the transcription factor NF-κB. To this purpose, we performed a Western blot analysis for p65 protein in the nuclear fraction of human endothelial cells under the experimental conditions described under Materials and methods. The results revealed that human endothelial cells grown under normal glucose conditions exhibited a low level of expression of p65 in the nuclear fraction (Fig. 3A, left lane). In contrast, endothelial cells cultured for 7 days in high glucose had a significantly increased (50%) level of NF-κB (Fig. 3A, middle lane, and B). The effect of high glucose on the translocation of NF-κB was significantly inhibited by treatment of the cells with enoxaparin, a situation in which the expression of NF-κB in the nuclear fraction was maintained at the control levels (Fig. 3A, right lane, and B).

3.2.3. Effect of enoxaparin on cellular reactive oxygen species

The level of intracellular reactive oxygen species was measured in human endothelial cells by fluorimetry using

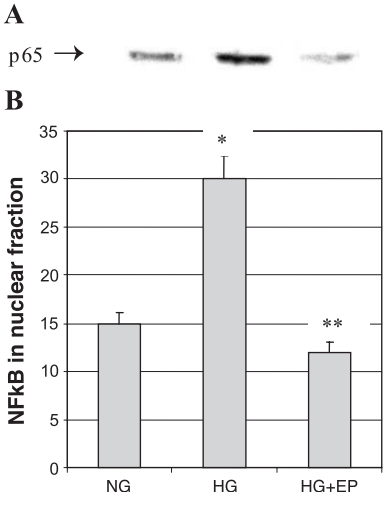

Fig. 3. (A) Representative Western blot displaying the expression of NF-κB in the nuclear fractions of HEC cultured in a normal (NG) glucose (left column), or a high glucose (HG) concentration (middle column) or in HG plus enoxaparin (HG + EP) in the culture medium (right column). (B) Quantitative densitometric evaluation of NF-κB detected by Western blot of the nuclear fractions of HEC. (*, significant different from control, $P<0.05$); (**, significantly different from HG, $P<0.05$).

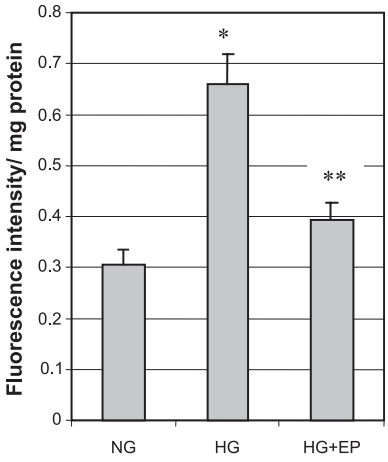

Fig. 4. Effect of enoxaparin on the intracellular level of reactive oxygen species (ROS) in HEC grown in the presence of a normal glucose (NG) or a high glucose (HG) concentration, or HG plus enoxaparin (HG + EP). Note that the ROS level was increased under HG conditions as compared to NG (*$P<0.05$) and that EP reduced the ROS level under HG conditions close to normal level (**$P<0.05$). The data are expressed as means ± S.E. of three independent experiments.

the dye DCF-DA, which freely permeates into cells and is hydrolyzed to fluorescent DCF upon interaction with reactive oxygen species, when present. In our experiments, as compared to cells cultured in the presence of a normal glucose concentration, the endothelial cells exposed for 7 days to a high glucose concentration exhibited a significant accumulation (two times higher) of intracellular reactive oxygen species (Fig. 4). Testing the effect of enoxaparin on the level of intracellular reactive oxygen species in human endothelial cells cultured under high glucose conditions, we found that 48 h of treatment with the drug suppressed the DCF fluorescence induced by high glucose in human endothelial cells to close to the control levels (Fig. 4).

4. Discussion

Previous studies indicate that an increased monocyte–endothelial cell interaction may play an important role in the accelerated atherosclerosis associated with diabetes. It has been shown that the binding of monocytes isolated from patients with Type II diabetes to cultured endothelial cells is higher than that to monocytes isolated from control, normal subjects (Carantoni et al., 1997; Kunt et al., 1999). Moreover, it has been demonstrated that monocyte adhesion to endothelial cells is increased when the latter are grown under high glucose conditions (mimicking the diabetic milieu) rather than in the presence of normal glucose concentrations (Kim et al., 1994; Morigi et al., 1998; Manduteanu et al., 1999). Among the possible mechanisms involved in this process, it has been suggested that endothelial cell adhesion molecules induced by the high glucose concentration may play a significant role (Morigi et al., 1998; Manduteanu et al., 1999).

Since we have previously found that the low-molecular-weight enoxaparin (clexane) has the ability to protect endothelial cells from activation induced by TNF-α or lipopolysaccharides, by inhibiting the expression of endothelial cell adhesion molecules and consequently monocyte adhesion, we performed experiments to establish whether enoxaparin interferes also with the endothelial cell activation induced by a high glucose concentration. We used cultured valvular endothelial cells, since it has been demonstrated that cardiac valves are a highly sensitive territory of the vascular system that, under hyperglycemic conditions (diabetes), exhibit rapidly the initial events occurring in atherogenesis, such as increased transcytosis of low-density lipoprotein (LDL) and monocyte adhesion, and develop atherosclerotic lesions at an accelerated rate (Simionescu et al., 1996). The concentration of enoxaparin used was 16 μg/ml, because this was the concentration that was calculated as a relevant plasma concentration. To approximate as close as possible the clinical situation, we studied the inhibitory properties of enoxaparin in concentrations in clinical use.

To study the effects of the drug on valvular endothelial cells exposed to a high glucose concentration, postconfluent cultured cells grown under high glucose conditions were exposed to a monocyte cell line (U937 cells) in the presence or absence of enoxaparin. We found that growing valvular endothelial cells in high glucose resulted in an increase in monocyte adhesion to the cells to about fourfold the control values. Also, the experiments revealed that treating postconfluent valvular endothelial cells with enoxaparin for 48 h diminished significantly monocyte adhesion to the cells (~ 45%).

Using an ELISA assay, we found that the mechanism underlying the effect of enoxaparin on the adhesive properties of endothelial cells involved a decreased expression of ICAM-1, VCAM-1, and E-selectin on the cell surface, indicating that enoxaparin interferes in valvular endothelial cell activation induced by a high glucose concentration.

To find out if these data apply to endothelial cells from other locations, we performed parallel experiments and tested the effect of enoxaparin on a human umbilical vein-derived EC line (the immortalized EA hy926 cells). The data indicated that enoxaparin had similar effects on the EA hy926 cells as on valvular endothelial cells, namely, the cells grown in high glucose plus enoxaparin had a less adhesive surface than the nontreated cells; moreover, the drug to depressed the expression of ICAM-1, E-selectin, and VCAM-1 on the surface of human endothelial cells.

Since the adhesion tests performed under similar conditions with valvular endothelial cells and human endothelial cells cultured in the presence of a high glucose concentration indicated an increased propensity of monocyte adhesion to valvular endothelial cells rather than human endothelial cells (fourfold and threefold, respectively, above the value obtained for cells grown in normal glucose), one can assume that valvular endothelial cells are more prone to high glucose-induced activation than human umbilical vein-derived endothelial cells. It has been reported that VCAM-1 is an important contributor to monocyte adhesion to endothelial cells (Jackson et al., 1999; Kalogeris et al., 1999). It is interesting to note that, in our experiments, when cells were grown under high glucose conditions, the level of VCAM-1 was higher on valvular endothelial cells than on human endothelial cells; this result is consistent with the increased adhesion of monocytes to valvular endothelial cells, and leads to the assumption that VCAM-1 is directly involved in the greater adhesion of monocytes to valvular endothelial cells than to human endothelial cells exposed to a high glucose concentration.

There is evidence that exposure of endothelial cells to a high glucose concentration induces an increase in NF-κB activity and that specific inhibitors of the NF-κB factor significantly reduce high glucose-induced enhanced monocyte adhesion. The data suggest that a high glucose concentration promotes upregulation of adhesive molecules through NF-κB activation (Morigi et al., 1998). To uncover the mechanism by which enoxaparin modulates ICAM-1, E-selectin and VCAM-1 expression, we performed experiments to test the effect of the drug on the activation of transcription factor NF-κB. We report here that growing endothelial cells in the presence of a high glucose concentration led to an increased expression of NF-κB in the nuclear fraction compared to the control level (cells grown under normal glucose conditions). Interestingly, when confluent cells were treated with enoxaparin, the drug reduced the p65 levels in the nuclear fraction to the control level. These results suggest that enoxaparin acts in the NF-κB activation pathway induced by high glucose conditions at an as yet unidentified level.

The mechanism(s) by which enoxaparin inhibits NF-kB activation is not clear. It has been shown that high glucose concentrations cause endothelial cell dysfunction through the generation of free radicals (Kashiwagi et al., 1996; Morigi et al., 1998), and that reactive oxygen intermediates induce NF-kB activation (Du et al., 1999; Kashiwagi et al., 1996). Our findings showing that growing endothelial cells under high glucose conditions result in an increased level of intracellular reactive oxygen species are consistent with these data. Under our experimental conditions, enoxaparin reduced reactive oxygen species levels to the control values. We may speculate that, like heparin, which has antioxidant properties (Finotti et al., 2001; Rahman et al., 1998), enoxaparin has the ability to decrease intracellular reactive oxygen species levels and may also diminish NF-kB activity, but further experiments are needed to establish a causal relationship between high glucose-induced reactive oxygen species generation, NF-kB and the blocking effect of enoxaparin.

Another potential site of enoxaparin action is protein kinase C (PKC), which is known to be involved in the deleterious effects of high glucose on endothelial cells (Ishii

et al., 1998). Since it has been shown that heparin interferes with the PKC pathway in smooth muscle cells (Herbert et al., 1996) and that this kinase is involved in the deleterious effects of high glucose on endothelial cells (Zhang et al., 2000), we may speculate that the drug can reduce the PKC activity induced by high glucose in endothelial cells. In our preliminary experiments, the Western blot analysis indicated that in endothelial cells subjected to high glucose conditions, enoxaparin reduced the amount of PKC translocated to the cell membrane fraction (unpublished data), but additional experiments need to be done to confirm this matter.

Taken together, our results indicate that enoxaparin has a beneficial effect on high glucose-induced endothelial cell activation (both on valvular endothelial cells and human endothelial cells) by reducing monocyte adhesion to endothelial cells via a mechanism involving the cell adhesion molecules—ICAM-1, VCAM-1, E-selectin—and the transcription factor NF-kB.

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