Heparin induces mobilization of osteoprotegerin into the circulation

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Summary
Heparin treatment may induce osteoporosis by an unknown mechanism. Osteoprotegerin (OPG), a glycoprotein with a heparin-binding site, is a decoy receptor for RANKL which is responsible for osteoclast development. The objective was to investigate the effect of unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH; dalteparin) on plasma levels of OPG. Twenty-two male students were allocated to the following treatment regimens: A) one bolus of 5,000 IU UFH iv followed by infusion of 450 IU/kg/24 h for 72 hours (n=7), B) sc administration of LMWH (200 IU/kg) once daily for 72 hours (n=8), C) sc administration of 100 IU/kg LMWH once (n=8), D) sc administration of 250 IU/kg UFH once (n=7), E) control infusion of saline for 12 hours (n=7). UFH boluses of 5,000 IU were given 4 and 24 hours after cessation of regimens A and B. Bolus injection of UFH iv caused a prompt increase in plasma OPG from 0.68 ng/ml (SD=0.09) to 1.13 ng/ml (SD=0.30) (p=0.003) which declined during the continuous UFH infusion and reached baseline values after 8 hours (regime A). Similar increases in plasma OPG was obtained by repeated UFH boluses after cessation of treatment. Subcutaneous administration of LMWH (200 IU/kg) caused a modest, but significant (p=0.002) increase in plasma OPG similar to the mobilization by 250 IU/kg UFH sc, but the LMWH treatment caused a three-fold higher anti-Xa activity (p<0.001). We conclude that UFH causes a more pronounced vascular mobilization of OPG than LMWH, indicating that UFH has a higher affinity for OPG than LMWH.

Keywords
UFH, LMWH, dalteparin, osteoprotegerin, osteoporosis

Introduction
Observations of spontaneous fractures in patients during long-term treatment with heparin in the 1960s suggested a possible relation between osteoporosis and heparin treatment (1). Later studies have confirmed that long-term use of heparin may cause osteoporosis and vertebral collapse following heparin treatment during pregnancy (2–4). Unfractionated heparin (UFH) may lead to symptomatic vertebral fractures in up to 3% and osteoporosis in approximately 30% of patients subjected to long-term heparin treatment (5). The pathogenesis of heparin-induced osteoporosis is not clear. Most studies performed in animals have shown that low-molecular-weight heparins (LMWH) have a lower propensity for inducing osteoporosis than UFH (6–8). In pregnant women, treatment with UFH adversely affected bone density (9, 10), whereas no differences in lumbar spine density were reported in pregnant women treated with dalteparin (11). Recently, a significantly lower bone mineral density (BMD) was reported in pregnant women randomized to treatment with UFH compared to treatment with dalteparin (12).

Osteoprotegerin (OPG) is a member of the tumour necrosis factor superfamily (13) which exerts its function as a soluble decoy receptor for receptor activator nuclear factor kappa β ligand (RANKL) (14). RANKL is necessary for development, maturation and activation of osteoclasts (14), and osteopetrosis is reported to develop in mice where OPG is transgenetically deleted (15). Moreover, a Fc-OPG construct administrated as one single subcutaneous (sc) injection reduced markers of bone turnover for a sustained period in postmenopausal women (16) and in women with multiple myeloma and breast cancer with skeletal metastases (17). Recent reports showed that antibodies to RANKL reduced bone resorption in patients with multiple myeloma and breast cancer with skeletal...
metastases (18) and increased bone mineral density in postmenopausal women with low bone mass (19). These findings, both in animals and humans, provided substantial evidence that the OPG/RANKL/RANK molecular triad has a crucial role in bone mass regulation.

OPG has a heparin-binding site in its carboxyterminal region (20). Interaction between OPG and the glucosaminoglycan heparan sulfate at the cell surface has been reported in myeloma cells (21) and in human monocytes (22). Recently, it was reported that binding of heparin to OPG inhibited OPG binding to RANK-RANKL complexes and that OPG reduced the half-life of membrane bound RANKL. (23). Thus, it was suggested that proteoglycans could be considered as essential co-factors modulating bone remodeling in favor of bone resorption (23). The aim of the present study was to investigate the impact of treatment with UFH and LMWH on plasma levels of OPG in humans.

Methods

Subjects
Twenty-two healthy, non-smoking, normolipemic male students at the University of Tromsø, who were consuming a traditional Western diet, were recruited to the study. Before inclusion, all participants underwent a clinical examination including a medical history and physical examination. Body weight, height, blood pressure, routine haematological and haemostatic variables (including platelet count, activated partial thromboplastin time [APTT], prothrombin time, and primary [Ivy] bleeding time) and routine biochemistry (including blood glucose, liver enzymes and serum lipids) were measured. Exclusion criteria were regular use of drugs, use of drugs that might interfere with haemostasis during the month prior to the study, general or local bleeding tendency, previous thromboembolic disease, arterial aneurysm, trauma or surgical treatment during the last month before inclusion, hypertension, hypersensitivity to heparin, liver or renal diseases, mental illness, and alcohol or drug abuse. Participants were instructed to refrain from strenuous physical exercise and alcohol for 48 hours (h) before each experimental protocol. The Regional Board of Research Ethics approved the study, and written informed consent was obtained from each individual.

Experimental design

The study was performed at the Clinical Research Centre at the University Hospital of Northern Norway. Technicians conducting analysis of samples collected from the study were blinded with regard to treatment regimen. Participants were randomly allocated to one or more of the following treatment regimens; A), intravenous (iv) bolus injection of 5,000 IU UFH (Heparin® 5,000 IU/ml, LEO Pharma AS) followed by a continuous infusion of UFH at an infusion rate of 450 IU/kg/24 h for 72 h (n=7); B), sc dalteparin (Fragmin®, Pfizer), 200 IU/kg given once daily for 72 h (n=8); C), sc dalteparin; 100 IU/kg given once (n=8); D) sc administration of 250 IU UFH given once (n=7); and E), continuous infusion of 500 ml isotonic saline for 12 h (n=7). After treatment regimens A and B, a second and third iv bolus injection of 5,000 IU of UFH were given at 76 and 96 h, that is 4 and 24 h after cessation of treatment. Subjects allocated to treatment regimen B are the same participants as in regimen C and E. They had a one-week washout period between each treatment regimen. In previous studies (results not shown) it was demonstrated that UFH in the given dosage iv and dalteparin 200 IU/kg given sc induced similar anticoagulant activity, measured as anti-Xa activity, over 12 h (24).

Blood sampling

Blood samples were collected immediately before each treatment regimen and at selected time intervals during the next 96 h. Blood for plasma preparation was collected into vacutainer tubes (Becton Dickinson, Meylan Cedex, France) containing 0.129 M sodium citrate (1 vol anticoagulant and 9 vol whole blood) through a 19-gauge needle or a permanent Venflon. Citrated plasma was prepared by centrifugation at 2,000xg for 15 minutes (min) at 22°C. Plasma samples were transferred into sterile cryovials in aliquots of 0.5 ml, and stored at –70°C until further analysis.

Assays

The concentration of total OPG (R&D Systems, Abingdon, UK) was measured by an ELISA assay according to the manufacturer’s instructions. The intra- and interassay coefficients of variation (CV) in our laboratory were 3.2% and 6.8%, respectively. The analyses were performed by a technician without knowledge of treatment regimen. All samples were measured in duplicate. Addition of 0–10 IU/ml UFH to plasma in vitro did not influence plasma OPG levels. sRANKL was analyzed by a new, highly sensitive ELISA assay for free RANKL with a detection limit for sRANKL of 0.02 pM (ampl sRANKL human, Biomedica, Vienna, Austria). The analysis was performed according to the manufacturer’s instructions. A Coulter Stack-S (Coulter Corp., Miami, FL, USA) was used for routine hemotological analysis, whereas serum enzymes, albumin, and creatinine were measured on a Hitachi 917 (Roche Diagnostics, GmbH, Mannheim, Germany) with reagents from the manufacturer. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured on an ACL 3000 coagulation analyzer (Nycodema Pharma-Cephotest® and Normotest®, respectively). Anti-Xa activity in plasma was measured with colorimetric assays for UFH (Rotachrom heparin, Diagnostica Stago) and LMWH (Rotachrom HBPM/LMWH, Diagnostica Stago) on a STA-Compact analyzer (Diagnostica Stago). The anti-Xa activity was calibrated with a commercial calibrator (STA-Heparin HBPM/LMWH, Diagnostica Stago) that was calibrated against the first international standard for LMWH or serial dilutions of UFH in reference plasma.

Statistics

Descriptive statistical analysis revealed normal distribution for the parameters studied. Continuous variables are presented as means (95% confidence interval, CI). Differences between means were tested for significance by paired t-test for comparison of variables within a treatment group, whereas an unpaired t-test was used for comparison of changes between treatment groups. Area under the curve (AUC) for anti-Xa activity was calculated according to the method described by Tai (25). Incremental AUC (AUCi) for OPG was measured to adjust for different basal values of OPG in the various study regimens. The AUC
and AUCi in the groups were compared by analysis of variance (GLM procedure). Where significant differences were found in allover analysis post-hoc tests were carried out (Bonferroni). The statistical analysis was performed using SPSS software for Windows, version 14.0 (SPSS Inc, Chicago, IL, USA). Two sided p-values < 0.05 were considered statistically significant.

Results

Characteristics of the participants are shown in Table 1. All participants exhibited normal haemostasis assessed by medical history and laboratory screening. No adverse events or bleeding complications were observed during the study.

To study diurnal variation in plasma OPG, 500 ml 0.9% saline was continuously infused in healthy volunteers (n=7) from 8 a.m. for 12 h with subsequent measurement of plasma OPG levels at selected time intervals (Fig. 1). Plasma OPG levels showed a modest, but significant decrease already after 30 min and remained decreased throughout the following 12 h and returned to baseline values the next morning.

Percent change in plasma levels of OPG after bolus heparin (5,000 IU) followed by continuous infusion of 450 IU/kg/24 h UFH are shown in Figure 1. Bolus heparin caused a prompt increase in plasma OPG levels from 0.68 ng/ml (SD±0.09) at baseline to 1.13 ng/ml (SD ±0.30) (p=0.003) after 5 min, followed by a gradual decrease during continuous UFH infusion (450 IU/kg/24 h) which reached baseline values 8 h after start of infusion. The corresponding anti-Xa activity is shown in Figure 1. Plasma OPG did not differ significantly from baseline throughout the rest of the 72 h continuous UFH infusion (data not shown). Plasma concentrations of sRANKL were not significantly changed by bolus injection (5,000 IU) or prolonged treatment with 450 IU/kg/24 h heparin (Fig. 2).

Sc administration of 250 IU UFH/kg and 200 IU dalteparin/kg caused modest 15–17% increase in plasma OPG concentrations (p=0.028 and p=0.002, respectively) with peak concentrations occurring at 2 h after administration. No difference in peak OPG levels was observed between groups. Plasma OPG levels returned to baseline values 12 h after both treatment regimens (data not shown). Subcutaneous administration of 100 IU dalteparin/kg did not affect plasma OPG (Fig. 3).

Incremental AUC (AUCi) for plasma OPG and AUC for anti-Xa activity for the different treatment regimens are shown in Table 2. The AUC for anti-Xa during treatment with 100 IU/kg dalteparin was set to 1, and the relative AUC for anti-Xa activities were calculated for the other regimens. There was a significant difference between the treatment regimens allover in AUCi

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Table 1: Characteristics of the healthy male volunteers participating in the study (n=22).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Means (95%CI)</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.8 (23.4–26.2)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>240 (21.6–26.3)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>144 (130–158)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>80 (72–88)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.9 (14.3–15.4)</td>
</tr>
<tr>
<td>Platelets (x10⁹/l)</td>
<td>221 (163–278)</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>28 (26.5–29.3)</td>
</tr>
<tr>
<td>Normotest (%)</td>
<td>97 (80–114)</td>
</tr>
<tr>
<td>Creatinine (µM)</td>
<td>87 (80–93)</td>
</tr>
<tr>
<td>Osteoprotegerin (pg/ml)</td>
<td>687 (612–762)</td>
</tr>
</tbody>
</table>

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Figure 1: Line graphs showing percent change from baseline in plasma OPG levels after a bolus dose of 5,000 IU UFH given intravenously followed by continuous infusion of UFH (450 IU/kg/24 h) (n=7) or during continuous infusion of saline (n=7) (upper panel). Corresponding anti-Xa activities are shown in the lower panel. Values are means ± SEM.

Figure 2: Line graph showing percent change from baseline in plasma sRANKL level after a bolus dose of 5,000 IU UFH given intravenously followed by continuous infusion of UFH (450 IU/kg/24 h) (n=5). Values are means ± SEM.
for plasma OPG (p<0.001) and for AUC for anti-Xa activity (p<0.001). The change in plasma AUCi for OPG did not differ between controls and after treatment with 100 IU/kg dalteparin (p=1.0). The AUCi for plasma OPG were higher for persons given dalteparin 200 IU/kg sc, UFH 250 IU/kg sc and UFH 450 IU/kg iv compared to controls (p=0.038, 0.041 and <0.001, respectively). The AUCi for plasma OPG for the group given UFH 450 IU/kg was also significantly higher compared to those treated with dalteparin 100 IU/kg (p<0.001), whereas for those given dalteparin 200 IU/kg and UFH 250 IU/kg the differences compared to the low-dose dalteparin almost reached statistical significance (p=0.084, p=0.088, respectively) (Table 2). No significant difference in anti-Xa activity was observed for dalteparin 100 IU/kg and UFH 250 IU/kg (Table 2). Despite that the anti-Xa activity was significantly lower during treatment with UFH 250 IU/kg sc compared to dalteparin 200 IU/kg (p<0.001) (Table 2), no significant difference in plasma OPG concentration was found between the two regimens. Although similar anti-Xa activity was observed during 8 h treatment with continuous UFH, nor treatment with sc dalteparin affected plasma mobilization of OPG by bolus heparin. Continuous treatment with UFH did not affect plasma OPG collected before bolus UFH, whereas preheparin plasma OPG 4 h after cessation of dalteparin treatment showed a 10% transient decrease (p=0.012).

Discussion

The present study is, to our knowledge, the first to show that both iv and sc administration of UFH causes mobilization of OPG into the circulation. Iv UFH promoted an immediate mobilization of OPG into the circulation with peak concentrations of OPG occurring already after 5 min and then gradually declined to baseline values within 8 h during continuous infusion of therapeutic doses with UFH (450 IU/kg/24 h) (Fig. 4, upper panel) or treatment with sc dalteparin 200 IU/kg given once daily (Fig. 4, lower panel). Bolus UFH caused 43–51% increase in plasma OPG. Neither treatment with continuous UFH, nor treatment with sc dalteparin affected plasma mobilization of OPG by bolus heparin. Continuous treatment with UFH did not affect plasma OPG collected before bolus UFH, whereas preheparin plasma OPG 4 h after cessation of dalteparin treatment showed a 10% transient decrease (p=0.012).

Figure 4 shows plasma OPG levels before and after bolus UFH (5,000 IU) at baseline and 4 and 24 h after cessation of 72 h continuous infusion with UFH (450 IU/kg/24 h) (Fig. 4, upper panel) or treatment with sc dalteparin 200 IU/kg given once daily (Fig. 4, lower panel). Bolus UFH caused 43–51% increase in plasma OPG. Neither treatment with continuous UFH, nor treatment with sc dalteparin affected plasma mobilization of OPG by bolus heparin. Continuous treatment with UFH did not affect plasma OPG collected before bolus UFH, whereas preheparin plasma OPG 4 h after cessation of dalteparin treatment showed a 10% transient decrease (p=0.012).

Figure 3: Line graphs showing percent change in plasma OPG from baseline after subcutaneous injection of high-dose LMWH (dalteparin, 200 IU/kg), low-dose LMWH (dalteparin, 100 IU/kg) and UFH 250 IU/kg (upper panel). Corresponding anti-Xa activities are shown in the lower panel. Values are means ± SEM.

Figure 3: Line graphs showing percent change in plasma OPG from baseline after subcutaneous injection of high-dose LMWH (dalteparin, 200 IU/kg), low-dose LMWH (dalteparin, 100 IU/kg) and UFH 250 IU/kg (upper panel). Corresponding anti-Xa activities are shown in the lower panel. Values are means ± SEM.

The cellular origin and the mechanism by which heparins promote OPG release into circulating blood is unknown. OPG is expressed at high concentrations by a variety of tissues and cell types including main components of the cardiovascular system such as arterial smooth muscle cells and endothelial cells (26, 27). Recently, it was reported that OPG is colocalized with vWF in Weibel Palade bodies in endothelial cells (28) and binds to glucosaminoglycans (GAGs) (i.e. heparin sulfate) at cellular membranes through its highly basic heparin binding domain (21–23). Lipoprotein lipase and tissue factor pathway inhibitor and other proteins in the vascular system with heparin-binding sites, are under normal conditions attached to GAGs by electrostatic forces at the endothelial surface (29, 30). Heparin treatment causes an immediate mobilization of these proteins into the circulation by displacement from the endothelial surface since they have higher affinity for heparins than GAGs at the endothelial surface (31, 32). Furthermore, secretion of TFPI from intracellular stores in endothelial cells also contributes to the prompt mobilization of TFPI by heparins (33). Previous studies have shown that OPG is synthesized and stored in endothelial cells (28), whereas the present study showed a prompt mobilization of OPG into the circulation similar to that reported for TFPI and
LPL. Thus, it is likely to assume that heparins mobilize OPG into the circulation by displacement from the endothelial surface or from intracellular stores. In our study, sc administration of high-dose LMWH caused similar mobilization to that of sc low-dose UFH of OPG into the circulation, but with a three-fold higher heparin concentration measured by anti-Xa activity. Molecular weight-dependent release reactions for TFPI and LPL by heparins have been reported (34). Thus, our findings may suggest a similar molecular size-dependent mobilization of OPG by heparins. However, further experimental studies are needed to clarify the cellular origin and actual underlying mechanisms for mobilization of OPG to the circulation.

The transient increase in plasma OPG by bolus heparin returned to baseline levels after 8 h with continuous infusion of heparin and remained at baseline levels during the remainder of the 72 h of treatment. This observation indicates that mobilization of OPG by heparins is accompanied by an adapted synthesis, mobilization into the vascular compartment, redistribution and elimination of OPG to maintain normal plasma levels. Furthermore, prolonged treatment with UFH and LMWH neither affected plasma levels of OPG after discontinuation of treatment, nor the ability of bolus UFH to mobilize OPG into the circulation. These findings indicate that retention of intravascular stores and plasma levels of OPG is of high priority to maintain normal homeostasis. Recently, it was reported that recombinant OPG binds heparin with high affinity (23), and OPG mobilized into the circulation by heparins will most probably form OPG-heparin complexes through interactions with the heparin-binding site as previously described for the interaction between TFPI and heparins. Heparin treatment is known to cause upregulation of TFPI synthesis and increase the release of TFPI in endothelial cells (35) with subsequent formation of TFPI-heparin complexes in the circulation which inhibited renal clearance of TFPI (34) and caused depletion of intravascular stores of TFPI (34).

Table 2: Incremental area under the curve (AUCi) for plasma osteoprotegerin (OPG) and area under the curve (AUC) for anti-Xa activity during the first 8 h of the different treatment regimens. Values are means (95% CI).

<table>
<thead>
<tr>
<th>Treatment (n)</th>
<th>OPG AUCi (ng/min/ml)</th>
<th>Anti-Xa activity AUC (U/min/ml)</th>
<th>Relative anti-Xa activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (7)</td>
<td>-31.7 (-63.5 – 0.2)</td>
<td>11 (48 – 69)</td>
<td></td>
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<tr>
<td>Dalteparin 100 IU/kg (8)</td>
<td>-23.0 (-52.8 – 6.8)</td>
<td>216 (157 – 275)</td>
<td>1 (0.73 – 1.27)</td>
</tr>
<tr>
<td>Dalteparin 200 IU/kg (8)</td>
<td>35.1 (5.3 – 64.9)</td>
<td>419 (364 – 474)</td>
<td>1.94 (1.69 – 2.19)</td>
</tr>
<tr>
<td>UFH 250 IU/kg sc (7)</td>
<td>36.7 (4.9 – 68.6)</td>
<td>142 (83 – 201)</td>
<td>0.66 (0.38 – 0.93)</td>
</tr>
<tr>
<td>UFH 450 IU/kg iv (7)</td>
<td>96.2 (64.3 – 128.0)</td>
<td>413 (354 – 472)</td>
<td>1.91 (1.64 – 2.19)</td>
</tr>
</tbody>
</table>

Posthoc tests (Bonferroni)

<table>
<thead>
<tr>
<th>OPG AUCi</th>
<th>anti-Xa AUC</th>
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<tbody>
<tr>
<td>Controls vs dalteparin 100 IU/kg sc p=1.0</td>
<td>Controls vs dalteparin 100 IU/kg sc p&lt;0.001</td>
</tr>
<tr>
<td>Controls vs dalteparin 200 IU/kg sc p=0.038</td>
<td>Controls vs dalteparin 200 IU/kg sc p&lt;0.001</td>
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<tr>
<td>Controls vs UFH 250 IU/kg sc p=0.041</td>
<td>Controls vs UFH 250 IU/kg sc p=0.031</td>
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<td>Controls vs UFH 450 IU/kg iv p=0.001</td>
<td>Controls vs UFH 450 IU/kg iv p=0.001</td>
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<tr>
<td>Dalteparin 100 IU/kg vs UFH 450 IU/kg p=0.001</td>
<td>Dalteparin 100 IU/kg sc vs UFH 450 IU/kg iv p=0.001</td>
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<tr>
<td>Dalteparin 100 IU/kg sc vs dalteparin 200 IU/kg sc p=0.084</td>
<td>Dalteparin 100 IU/kg sc vs dalteparin 200 IU/kg sc p&lt;0.001</td>
</tr>
<tr>
<td>Dalteparin 100 IU/kg sc vs UFH 250 IU/kg sc p=0.088</td>
<td>Dalteparin 100 IU/kg sc vs UFH 250 IU/kg sc p=0.086</td>
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<tr>
<td>Dalteparin 200 IU/kg sc vs UFH 250 IU/kg sc p=1.0</td>
<td>Dalteparin 200 IU/kg sc vs UFH 250 IU/kg sc p&lt;0.001</td>
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<tr>
<td>Dalteparin 200 IU/kg sc vs UFH 450 IU/kg sc p=0.075</td>
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</tr>
<tr>
<td>UFH 250 IU/kg sc vs UFH 450 IU/kg iv p=0.113</td>
<td>UFH 250 IU/kg sc vs UFH 450 IU/kg iv p&lt;0.001</td>
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</table>
entiation and activity (39,40), osteoclast number and activity (7) and bone resorption (6–8). However, the exact mechanism by which heparins induce osteoporosis is not known. Several studies have shown that OPG and RANKL are important for osteoclast activity and the balance between bone resorption and bone formation (13,14,41,42). Our findings indicate that UFH has higher affinity for OPG than LMWH within the vascular compartment. Furthermore, UFH treatment has been reported to be accompanied by accumulation of heparin in bone tissue in rats, which was retained for a long time after discontinuation of treatment (43). Thus, it may be speculated that UFH induces osteoporosis either by attracting OPG to the vascular compartment or by attenuating its function in the bone microenvironment.

Previously, it has been proposed that overactivation of osteoclasts by parathyroid hormone (PTH) or secondary increase of PTH caused by lowering of calcium in blood due to heparin binding of calcium are possible mechanisms for heparin-induced osteoporosis (44). Experimental (14,45) and clinical studies (42) have shown that continuous PTH increases RANKL and inhibits OPG expression and secretion even within the bone microenvironment (42), whereas intermittent PTH exposure has anabolic effects (46). Thus, PTH may either be directly involved in the pathophysiology of heparin-induced osteoporosis or indirectly by affecting the OPG-RANKL system.

In summary, our study showed that both UFH and high-dose LMWH (dalteparin) increase plasma OPG levels. Sc administration of high-dose LMWH increased the plasma OPG concentration similar to that of low-dose UFH, but was accompanied by three-fold higher anti-Xa activity. Iv administration of UFH is superior to sc administration of LMWH to mobilize OPG into the circulation. Treatment with UFH and LMWH for 72 h did not promote depletion of intravascular OPG. Our findings indicate that UFH has higher affinity for OPG than LMWH within the vascular compartment. Thus, it may be speculated that UFH induces osteoporosis either by attracting OPG to the vascular compartment or by attenuating its function in the bone microenvironment. Further clinical and experimental studies are warranted to investigate the role of the OPG-RANKL system in heparin-induced osteoporosis.

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