Low-Molecular-Weight Heparin (Dalteparin) Demonstrated a Weaker Effect on Rat Bone Metabolism Compared with Heparin

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ABSTRACT—We studied the pharmacological effects of dalteparin (low-molecular-weight heparin) and heparin on bone metabolism in rats. After their 28 days of consecutive intravenous injections, significant loss of bone weight and mineral contents was observed in the heparin-treated rats, whereas dalteparin slightly reduced bone mass. By the end of the experiment, the femora of 7 out of 8 rats fractured in the heparin (10,000 U/kg/day)-treated group, but none had broken in the control and dalteparin-treated groups. Serum osteocalcin levels were significantly decreased in the former group. The growth plate width of the tibia was increased in a dose-dependent manner, especially in the heparin-treated group. Histomorphometric assessment of tibia showed that the osteoid surface and mineral apposition rates were significantly reduced in the heparin-treated group, whereas the eroded surface was significantly increased in the heparin-treated group. The above results suggest that heparin not only augments bone resorption but also suppressed bone formation and that dalteparin has a weaker suppressive effect on bone formation compared with heparin.

Keywords: Heparin, Dalteparin sodium, Bone metabolism, Osteopenia

Heparin has been used clinically since the middle of the 1930's, and its efficacy in the treatment of thrombosis is well-documented. However, the clinical use of heparin is certainly accompanied with problems such as inhibition of platelet aggregation and lipolysis exertion. Furthermore, several observations (1–4) have confirmed that the long-term use of heparin as an anticoagulant may result in the development of clinical osteoporosis. Tarquini et al. (5) demonstrated that low doses of calcium heparin for 2 weeks are capable of inducing a significant increase in urinary hydroxyproline as a bone resorption marker in patients. Osteoporosis is a great problem in elderly people. In a rat model, high doses of heparin (2,000 U/kg/day for 2 weeks) induced significant bone loss (6). Another form of heparin used clinically is dalteparin (low-molecular-weight heparin, LMWH; M.W. 4,000–6,000), which has high antithrombin III (AT III) affinity without, unlike heparin, a significant enhancing effect on antithrombin activity (7). Recently, a significant difference in the development of spinal fractures was recognized between heparin and dalteparin therapies in elderly patients: the spinal fractures occurred in 5 out of 12 patients treated with heparin, but did not in 11 patients taking dalteparin (8). These findings suggest that dalteparin seems to be a good alternative to heparin, especially for elderly patients. To elucidate the mechanism of the advantageous effect of dalteparin, the present study was carried out to compare the effects of heparin and dalteparin on bone metabolism in vivo in rats.

MATERIALS AND METHODS

Chemicals
Dalteparin sodium (dalteparin, Fragmin®) was purchased from Pharmacia Pharmaceutical Co., Ltd., Uppsala, Sweden. The other chemicals used were obtained from the following sources: Heparin sodium salt (heparin), Sigma Chemical Company, St. Louis, MO, USA; oxytetracycline hydrochloride, Wako Pure Chemical Co., Ltd., Osaka; and calcein, Dojindo Co., Ltd., Kumamoto.

Animals
Six-week-old male S.D. rats (Japan SLC, Shizuoka) were housed individually in a room with a 12 hr/12 hr light/dark cycle and 60±10% humidity at a constant temperature (22±1°C). They were fed with a fixed amount of commercial diet (CE-2; Japan Clea, Tokyo) containing 1.2% calcium and 1.1% phosphorus and 2.0
I.U./g vitamin D and provided with distilled water ad libitum.

**Drug treatment**

Dalteparin or heparin (anti-factor Xa activity, 1,000, 3,000 and 10,000 U/2 ml/kg) dissolved in physiological saline was intravenously injected into a tail vein once a day for 28 days. The control rats were given saline as the vehicle. Bone labelings with tetracycline (25 mg/kg) and calcine (15 mg/kg) were performed once for each label at 8 and 4 days, respectively, before the end of the experiment.

**Method for bone roentgenography and microdensitometric analysis**

The rats were sacrificed under ether anesthesia at 24 hr after the last administration. Their femora were then removed and separated from surrounding soft connective tissues. As soon as the femoral dissection had been completed, roentgenograms were taken on a graphic art film (type CS 100E; Konica Co., Ltd., Tokyo) with a soft X-ray machine (Type SRO-505; Sofron Co., Ltd., Tokyo). An aluminum step wedge of 0.5-mm thickness was placed on the film beside each bone as a density standard, and microdensitometric analysis was then performed. Following the method of Yamazaki and Yamaguchi (9), the density of transverse sections of the femora was scanned from the point 0.18 to that 0.43 from the distal end of the bone (total length of the bone was taken as 1.0) on the roentgenogram by a computerized Flexible Image Processor (Model PIP-400; ADS Co., Ltd., Nara). Bone density (2GS/D) was also calculated by this machine, where D is the outer diameter of the femur, and 2GS is the area under the curve of the density chart from the X-ray film. Also, the cortical thickness index (CTI = (D - d) / D, d is inner diameter) was measured. Fracture of the femoral bone was also determined from the X-ray films.

**Determination of breaking strength**

Immediately after the roentgenography of femora, the breaking strength, the amount of strength required to horizontally break the femora, was measured with a Breaking Property Analyzer (Rheodynacorder, Model RDR-1500; Iio Electric Co., Ltd., Tokyo) (10).

**Determination of bone weight and mineral content**

The right femur and the 4th lumbar vertebra were rinsed twice in a chloroform : methanol (2 : 1) solution and then dried. They were weighed (dry weight) and thereafter heated in an electric furnace at 250°C, 400°C and 600°C for 30 min of each temperature and then at 700°C for 16 hr. Then, the ash weight was determined, after which the ash was dissolved in 1 N hydrochloride solution and its calcium and inorganic phosphorus contents were determined by commercial kits, Calcium C-test Wako™ and Phospha B-test Wako™ (Wako Pure Chemical Co., Ltd.), respectively.

**Biochemical analysis of serum**

At the end of the experiment, blood was obtained from the abdominal aorta under ether anesthesia. Serum calcium (cresolphthalein complexone method), inorganic phosphorus (phosphomolybdate method), total protein (biuret method), albumin (bromocresol green method), creatinine (Jaffe method) and alkaline phosphatase (ALP, Bessy-Lowry method) were determined with an autoanalyzer (RA-100; Japan Technicon, Tokyo).

**Determination of serum TGF-β1 and osteocalcin**

Serum transforming growth factor-β1 (TGF-β1) and osteocalcin were determined with commercial RIA kits: the former with a 125I-radioimmunoassay kit (DuPont/NEN Research Products, Boston, MA, USA) and the latter with rat osteocalcin radioimmunoassay reagents (Biomedical Technologies Inc., Stroughton, MA, USA).

**Histology**

At the end of the experiment, tibiae from each group were fixed in 10% formalin (neutral buffer) after the adherent soft tissues had been trimmed away, and the samples were then embedded in methyl metacylate (MMA). Serial nondecalcified 4-μm-thick frontal sections were prepared with a Reichert-Jung microtome (Model Poly-cut S; Leica, Heidelberg, Germany).

**Bone histomorphometry**

Histomorphometry was performed with a semiautomatic image-analyzing system linked to a light microscope (Optiphot 2; Nikon, Tokyo). In the metaphyseal region, a cancellous bone area more than 1-mm distal from the growth plate was observed. Villanueva-stained MMA sections were measured to determine trabecular bone volume (bone volume / tissue volume, BV/TV, %), osteoid surface (osteoid surface / bone surface, OS/BS, %), osteoclast number (Oc.N/BS, mm⁻¹), osteoclast surface (Oc.S/BS, %) and eroded surface (ES/BS, %). Mineral apposition rate (MAR, μm/day) was determined by the value of the width between double labels.

**Statistical analysis**

Results were expressed as the mean ± standard error of the mean (S.E.). Statistical significance of the difference between the control and drug-treated groups was determined by Dunnett’s multiple test or χ²-test and that between heparin- and dalteparin-treated groups, by Student’s t-test.
Table 1. Effects of dalteparin and heparin on body weight and bone femur length in male rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (U/kg/day)</th>
<th>Body weight (g) Initial</th>
<th>Final</th>
<th>Femur length (cm)</th>
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<tr>
<td>Control</td>
<td></td>
<td>197.8±1.3</td>
<td>226.6±4.0</td>
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<tr>
<td>Dalteparin</td>
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<td>3.47±0.03</td>
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<tr>
<td></td>
<td>3000</td>
<td>198.4±1.5</td>
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<tr>
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<td>10000</td>
<td>199.6±0.7</td>
<td>229.9±3.5</td>
<td>3.52±0.03</td>
</tr>
<tr>
<td>Heparin</td>
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<td>227.4±3.7</td>
<td>3.50±0.02</td>
</tr>
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<td>199.4±1.1</td>
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</tr>
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<td>199.9±1.8</td>
<td>224.7±3.7</td>
<td>3.45±0.02</td>
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Rats were intravenously administered dalteparin or heparin for 28 days. The control rats were given physiological saline as the vehicle for 28 days. Values are the mean±S.E. of 8 animals.

Table 2. Femoral fracture rates in dalteparin- and heparin-treated male rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (U/kg/day)</th>
<th>Fracture rate (per 8 rats)</th>
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<td>Unilateral</td>
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<td>Control</td>
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</tr>
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<td>Dalteparin</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10000</td>
<td>0</td>
</tr>
<tr>
<td>Heparin</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10000</td>
<td>1</td>
</tr>
</tbody>
</table>

Rats were intravenously administered dalteparin or heparin for 28 days. The control rats were given physiological saline as the vehicle for 28 days. Values are the femoral fractures observed, expressed as the number of animals with fractures per 8 rats in the group. *: Significantly different from the control at P<0.05.

RESULTS

Body weight and bone analysis

The body weight and femoral bone length of the dalteparin- and heparin-treated rats were similar to those of the control 28 days after the start of the experiment (Table 1). Fractures in femora were not seen in the control or dalteparin-treated rats, but fractures of the unilateral femur were exhibited in 1/8 of the rats treated with heparin at 1,000 U/kg/day and in 2/8 of those given heparin at 3000 U/kg/day. In rats administered the highest dose of heparin (10,000 U/kg/day), 1/8 of the rats had fracture of the unilateral femur and 6/8 had fractures of bilateral femora at the end of the experiment (Table 2). Figure 1 shows typical roentgrams of distal femora. At the end of 28 days of treatment with dalteparin or heparin, breaking strength and metaphyseal bone density (ΔG/S/D) determined by microdensitometric analysis were dose-dependently decreased. Compared with dalteparin-treated rats, heparin-treated ones showed more strongly reduced metaphyseal bone density. Also, heparin (3,000 U/kg/day) and dalteparin (10,000 U/kg/day) decreased the CTI significantly (Table 3 and Fig. 1).

Fig. 1. Typical roentgrams of male rat distal femora. Rats were administered dalteparin or heparin (10,000 U/kg/day, i.v.) for 28 days. The control rats were given physiological saline as vehicle for 28 days. A. Control, B. Dalteparin, C. Heparin. bar: 1 mm.
Rats were intravenously administered dalteparin or heparin for 28 days. The control rats were given physiological saline as the vehicle for 28 days. Values are the mean ±S.E. of 8 animals, except for heparin at 10,000 U/kg/day, where n = 2 (no fractures observed). 2GS/D: bone density; D = outer diameter of femora, 2GS = area under the curve of the X-ray film density chart. CTI: cortical thickness index; (D - d) / D, d is inner diameter. * **: Significantly different from the control at P <0.05, P <0.01, respectively.

Dry and ash weights, and calcium and phosphorus contents of the femur and the 4th lumbar vertebra

The dry weight of the femur and the 4th lumbar vertebra was decreased in both dalteparin- and heparin-treated groups at their highest dose (Table 4). The dry weight of femora was significantly decreased in dalteparin- and heparin-treated groups compared with the control weight at the highest dose (10,000 U/kg/day). For the 4th lumbar vertebra, a significant change was seen only in the heparin (10,000 U/kg/day) group. Ash weights for the femur and the vertebra showed a pattern of change similar to those for the dry weight. For both dry and ash weights of the femur and vertebra, heparin decreased those weights more markedly than dalteparin (Table 4). Calcium and phosphorus contents in the femur and the 4th lumbar vertebra were decreased in parallel with the changes in ash weights (Fig. 2). There is a good correlation between Ca or P contents and ash weights in not only the heparin-treated groups but also in the dalteparin-treated groups. At the dose of 10,000 U/kg, heparin significantly reduced calcium and phosphorus contents compared with dalteparin.

Biochemical analysis of serum

After 28 times daily doses of either drug, the serum calcium content was similar to that of the control rats, but serum phosphorus was significantly increased in heparin (10,000 U/kg/day)-treated rats. Neither dalteparin nor heparin had any effect on serum ALP activity or total protein in the serum. However, serum albumin concentrations were dose-dependently decreased in the heparin-treated rats, resulting in a drop in the albumin/globulin concentration ratio (A/G) in the heparin-treated rats (Table 5).
Fig. 2. Effects of dalteparin and heparin on calcium (A) and phosphorus (B) contents of femora and the 4th lumbar vertebrae of male rats. Rats were intravenously administered dalteparin or heparin for 28 days. The control rats were given physiological saline as vehicle for 28 days. Each column represents the mean ± S.E. of 8 animals. *, **: Significantly different from the control at P<0.05, P<0.01, respectively. #, ##: Significantly different from the respective dose of dalteparin at P<0.05, P<0.01, respectively.

Table 5. Effects of dalteparin and heparin on serum calcium, phosphorus, alkaline phosphatase (ALP) activity, total protein, albumin and albumin/globulin (A/G) in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (U/kg/day)</th>
<th>Calcium (mg/dl)</th>
<th>Phosphorus (mg/dl)</th>
<th>ALP (U/l)</th>
<th>T. Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dalteparin</td>
<td>1000</td>
<td>10.0±0.2</td>
<td>7.4±0.3</td>
<td>196.9±18.4</td>
<td>6.20±0.12</td>
<td>3.47±0.05</td>
<td>1.29±0.06</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>10.2±0.1</td>
<td>7.5±0.2</td>
<td>183.2±11.9</td>
<td>6.27±0.10</td>
<td>3.41±0.04</td>
<td>1.21±0.05</td>
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<tr>
<td></td>
<td>10000</td>
<td>10.5±0.1</td>
<td>8.5±0.4</td>
<td>211.6±18.4</td>
<td>6.11±0.06</td>
<td>3.23±0.05</td>
<td>1.13±0.04</td>
</tr>
<tr>
<td>Heparin</td>
<td>1000</td>
<td>10.2±0.2</td>
<td>8.3±0.4</td>
<td>170.7±8.6</td>
<td>6.17±0.11</td>
<td>3.17±0.06*</td>
<td>1.06±0.04*</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>10.4±0.2</td>
<td>7.9±0.2</td>
<td>164.4±17.0</td>
<td>6.24±0.13</td>
<td>3.06±0.08**</td>
<td>0.98±0.07**</td>
</tr>
<tr>
<td></td>
<td>10000</td>
<td>10.3±0.1</td>
<td>8.7±0.3*</td>
<td>203.1±25.0</td>
<td>6.33±0.14</td>
<td>2.99±0.09***</td>
<td>0.91±0.06***</td>
</tr>
</tbody>
</table>

Rats were intravenously administered dalteparin or heparin for 28 days. The control rats were given physiological saline as the vehicle for 28 days. Values are the mean±S.E. of 8 animals. *, **: Significantly different from the control at P<0.05, P<0.01, respectively. *: Significantly different from the respective dose of dalteparin at P<0.05 and P<0.01, respectively.
Effects of dalteparin and heparin on serum TGF-β1 (A) and osteocalcin (B) concentrations in male rats. Rats were intravenously administered dalteparin or heparin for 28 days. The control rats were given physiological saline as the vehicle for 28 days. Each column represents the mean ± S.E. of 8 animals. *: Significantly different from the control at $P < 0.05$, $P < 0.01$, respectively. #: Significantly different from the respective dose of dalteparin at $P < 0.05$.

**Serum TGF-β1 and osteocalcin concentration**

The serum TGF-β1 level in dalteparin- or heparin-treated rats showed a tendency to increase, and the increase over the control level was significantly different at the dose of 10,000 U/kg/day (Fig. 3A). The serum osteocalcin level was decreased dose-dependently in heparin-
treated rats. The osteocalcin level in the heparin (10,000 U/kg/day)-treated rats was significantly lower compared with that of the control and dalteparin-treated rats (Fig. 3B).

**Bone histomorphometry**

The tibial growth plate width was increased in a dose-dependent manner, and significant changes from the control value were observed in dalteparin (10,000 U/kg/day)- and heparin (3,000 U/kg/day)-treated groups (Fig. 4A). The OS/BS was dose-dependently reduced in the heparin-treated groups, and the reduction from the control was significant at the dose of 3,000 U/kg/day. Although the ES/BS was significantly increased in the heparin-treated rats, the osteoclast surface was not significantly changed from the control in these animals. However, the Oc.N/BS was significantly increased in heparin-treated groups but not in dalteparin-treated ones (Fig. 4B). The tibias of the heparin (10,000 U/kg/day)-treated group were very fragile, and thus frontal sections could not be obtained.

**DISCUSSION**

Dalteparin is a LMWH (M.W. 4,000–6,000) that has high AT III affinity without, unlike heparin, a significant enhancing effect on antithrombin activity (11). Compared with heparin, dalteparin possesses several advantages such as longer antifactor Xa activity and less hemorrhagic side effects in humans (12). Shibata et al. reported earlier that no fracture was seen in rat femora after dalteparin injection for 28 days, even at the high dose of 80,000 U/kg/day (personal communication). Therefore, the effect of dalteparin on bone metabolism can be considered to be essentially different from that of heparin. Consequently, from the viewpoint of an alternative for heparin, this study was aimed at elucidating the advantageous mechanism of dalteparin on the bone metabolism.

The present study in rats demonstrated that dalteparin or heparin treatment can significantly induce bone loss determined by mineral contents. Especially, heparin strongly reduced the bone mineral density in this animal. The magnitude of bone mineral loss induced by dalteparin at 10,000 U/kg/day was similar to that by heparin at 3,000 U/kg/day. However, fracture of the femur was seen in neither saline- nor dalteparin-treated rats, whereas dose-dependent spontaneous fractures were caused in heparin-treated rats.

As osteocalcin is a basic protein synthesized in and secreted from osteoblast cells, its concentration in serum represents a grade marker of bone formation (14). In the present study, serum osteocalcin concentration was decreased in heparin-treated rats. Furthermore, histomorphometric analysis disclosed that the OS/BS was significantly reduced in the heparin-treated rats, but well maintained in the dalteparin-treated rats. These results indicate that heparin strongly suppresses bone formation.

Heparin has been reported to increase the production of collagenase in mouse calvaria cultures (15). It is also known that osteoblasts produce collagenase in response
to bone resorption-stimulating hormone (parathyroid hormone), whereas osteoclasts do not (16). So these results suggest that heparin can stimulate osteoblasts activity. As shown by the histomorphometric analysis, the difference in action on bone metabolism between heparin and dalteparin is mainly based on the degree of suppression of ossification. We conclude that this suppressive effect of heparin on the bone-forming ability of the osteoblast is an important factor leading to bone fractures.

In this study, heparin decreased serum albumin level dose-dependently. On the contrary, coincubating heparin with collagen stimulated albumin synthesis in rat hepatocyte culture (17). The interaction heparin with collagen was reported to be important for maintenance of albumin synthesis (17), but heparin was also reported to inhibit collagen synthesis in fetal rat calvaria culture (18). So heparin may modulate albumin synthesis by depression of collagen synthesis.

Chowdhury et al. (13) reported that neutralization of the negative charges on heparin completely removed the stimulatory effects of this molecule on osteoclast activity. Unfractionated heparin comprises structurally heterogeneous sulfated polysaccharides. Lam et al. (19) and Hock et al. (20) reported that porcine heparin could be fractionated into two fractions (high affinity and low affinity) with equal yields according to its AT III affinity. Shimotori and Sakuragawa (21) reported that dalteparin was separable into three fractions, high affinity, low affinity and no affinity: only 26% of the total dalteparin was able to bind AT III with high affinity, and the low and no affinity fractions were 39% and 35%, respectively. However, in the rat model using progressive occlusive damage, dalteparin produced an antithrombotic effect similar to heparin (22). These investigations demonstrate that dalteparin is composed of different fractions compared to heparin. There is evidence to suggest that different sequences of heparin may be responsible for different biological activities (23).

Another LMWH (logiparin) was reported to induce osteoporosis in rats to the same degree as heparin, if it is dose-adjusted, as same factor Xa inhibitory activity to heparin (24). The mean molecular weight of logiparin resembles that of dalteparin, but its antifactor Xa activity/weight is lower than that of dalteparin (24). In healthy subjects study, dalteparin showed significantly higher antifactor Xa peak activity and area under the curve than those of logiparin after subcutaneous injection (25). To achieve identical antifactor Xa activity as dalteparin, logiparin must be administered at an 80% higher amount than dalteparin (24). Such an increase in the weight of administered logiparin also increases the amount of glycosaminoglycans that have non-antifactor Xa activity. These fractions, having factor Xa binding ability, may induce osteoporosis. Heparin is also recognized as an important regulator of cell growth (26). The antiproliferative activity of heparin on vascular smooth muscle cells is reported to be independent of its ability to bind AT III (27). It is reasonable to assume that there is no correlation between AT III binding ability or the factor Xa inhibitory activity and induction of osteoporosis, like the antiproliferative activity of heparin on vascular smooth muscle cells.

In fetal rat calvaria culture, LMWHs (dalteparin, logiparin, enoxaparin and ardeparin) produce significantly less calcium loss than heparin (28), and no significant difference was observed between these LMWHs in terms of their ability to promote 45Ca release (28). Thus, molecular size is suggested to be a major determinant of heparin's ability to stimulate bone resorption. Logiparin showed a weaker stimulative effect on bone resorption, but it was also reported to decrease bone density to similar extent as heparin (24). These findings suggest that decreased bone formation rates is a major determinant of heparin-induced osteoporosis. So, it is very important to determine whether there is a difference between dalteparin and heparin with respect to their effects on bone metabolism.

In fetal rat calvaria cultures, heparin was reported to inhibit the incorporation of 3H-proline into collagenase-digestible protein, and this effect was additive to the inhibitory effect of basic fibroblast growth factor (bFGF) (18). Nagai et al. reported that bFGF increased the growth plate width and decreased the rate of bone formation in rats (29). In the present study, heparin also widened the growth plate and suppressed the bone formation rate. Furthermore, bFGF has been reported to stimulate the proliferation of chondrocytes and inhibit their terminal differentiation and calcification (30). It is known that heparin or heparan sulfate is required for the binding of bFGF to its high-affinity receptor (31). Depending on the tissue or cell type, heparin can either stimulate or inhibit cell proliferation, and some of these effects may be mediated by bFGF (32). Heparin and heparan sulfate have been shown to protect bFGF from degradation by heat, acid and proteolysis (33), and these compounds are required to form a stable bFGF complex in the absence of other cell surface molecules (34). So heparin may also depress calcification by stabilizing bFGF.

Heparin binding sites are found in many other proteins. Human bone cell attachment to heparin-binding sites of fibronectin are necessary for their spreading in culture (35). This attachment is inhibited by heparin (35). Bone morphogenetic protein 2 is a triggering factor of bone formation. Also, bone morphogenetic protein 2 has...
heparin-binding sites, and its activity is increased by heparin (36). It is postulated that heparin-binding sites act to localize these proteins by restricting their diffusion. It is reported that different cytokines interact with extracellular matrix components (37). Yang and Yang reported that heparin treatment of bone marrow stroma fibroblast cell cultures decreased their synthesis of interleukin-11 and granulocyte macrophage colony-stimulating factor mRNAs (38). These studies suggest that heparin may modulate bone metabolism by interaction with those biological active proteins. However, the mechanism of heparin-induced osteopenia is complex due to the multiple biological activities of heparin. Therefore, further studies are needed to elucidate the mechanism by which heparin depresses osteoblast function and differences between heparin and dalteparin.

In conclusion, our data have shown a stimulatory effect of heparin on bone resorption and a suppressive effect on bone formation in the rat. Also, dalteparin demonstrated a weaker effect on bone resorption and formation, with the greatest difference in heparin and dalteparin being the degree of their suppressive action toward osteoblasts. According to these findings, the osteoblast appears to have a main role in heparin-induced osteopenia. In clinical applications (39, 40), dalteparin was usually used at half or one quarter the dosage of heparin for prophylaxis of venous thrombosis in humans. From these results, the risk of bone fracture may be reduced by alternative usage of dalteparin instead of heparin.

Acknowledgments
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