Effect of Recombinant Human Granulocyte Colony-Stimulating Factor (rh G-CSF) on Rat Bone: Inhibition of Bone Formation at the Endosteal Surface of Vertebra and Tibia

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Abstract. The effect of recombinant human granulocyte colony-stimulating factor (rh G-CSF) on bone was evaluated by histomorphometry using Sprague-Dawley rats. rh G-CSF was injected at doses of 0, 50, 150, and 450 μg/kg for 6 weeks. In vivo double fluorochrome labeling was performed before sacrifice. No significant change in body weight was observed. Bone mineral density (BMD) of lumbar vertebrae and femora was significantly decreased in G-CSF-treated groups. In the lumbar vertebra, osteoid surface, osteoid thickness, trabecular thickness, and labeled surface in G-CSF-treated groups were also significantly lower. In addition, osteoclast number and osteoclast surface were significantly higher in the G-CSF-treated groups. The endocortical surface at the mid-tibia showed lower labeled surface and mineral apposition rate in G-CSF-treated groups, without significant changes at the periosteal surface. Furthermore, numerous granulocytes fully occupied the bone marrow area. We conclude that proliferating granulocytes in the bone marrow may inhibit bone-forming cells from contacting the bone surface, resulting in reduction of bone formation; and increased osteoclastic bone resorption induced by G-CSF treatment contributed to the reduction of BMD.

Key words: G-CSF — Bone histomorphometry — BMD — Rat — In vivo.

It is well known that recombinant human granulocyte colony-stimulating factor (rh G-CSF) has specific actions on neutrophils and granulocyte progenitor cells. Therefore, recently rh G-CSF has been used widely for treatment of bone marrow suppression after chemotherapy and irradiation for malignant diseases and neutropenia. Bone pain has occasionally been observed in patients treated with high doses of G-CSF. Furthermore, toxicity studies of G-CSF in rats have revealed histologic changes in long bones and vertebrae [1, 2], suggesting that G-CSF has bone effects. However, there are not reports that examine G-CSF effects on bone, particularly in a dose-effect fashion. The purpose of this study was to characterize the effect of G-CSF on bone in rats.

Materials and Methods

The experimental animals were 6-week-old male, Sprague-Dawley rats (Nippon Charles River Co. Ltd., Yokohama, Japan), each weighing approximately 150 g at the beginning of the study. Twenty-four rats were divided into five groups and treated with different doses of rh G-CSF (Kirin-A Amgen Co. Ltd., Tokyo, Japan). The injection solution was prepared in a vehicle of 10 mM acetate acid buffer as follows: control, vehicle, 50 μg/kg, 150 μg/kg, and 450 μg/kg. The rats were randomly distributed to groups by initial weight and weighed weekly. The doses of G-CSF were weight adjusted each week. All rats were housed in group cages and fed a commercial diet (Oriental Yeast Co. Ltd., Tokyo, Japan) containing 1.15% calcium, 0.88% phosphorus, and 80 IU/100 g vitamin D₃. These rats were given food and water ad libitum during the experimental period. Subcutaneous injections of vehicle and rh G-CSF were given 6 days a week for 6 weeks. The rats received a single dose of calcine (3’-3’-bis[N,N’-di(carboxymethyl)aminomethyl]fluorescein) 15 mg/kg subcutaneously on the sixth day before autopsy, and a single dose of oxetetracycline 20 mg/kg intravenously on the second day before autopsy. At sacrifice, the sixth lumbar vertebrae, right femora, both tibiae, and serum were obtained. Bone mineral density (BMD), bone histomorphometry, and biochemical parameters in the serum (Ca, Pi, Alk-p, PGE, osteocalcin) were measured. BMD of the sixth lumbar vertebra and right femur were first measured using a Hologic QDR-1000 X-ray Bone Densitometer (Hologic Inc., Waltham, MA) in ultrahigh resolution mode. Then, the sixth lumbar vertebra and right tibia were fixed in 70% alcohol and embedded in methacrylate without decalcification after Villanueva bone stain. The specimens of vertebrae were cut perpendicular to both endplates at the midsagittal line and 5 μm-thick parasagittal sections were obtained using Jung K Microtome (Carl Zeiss, Heidelberg, Germany). Bone histomorphometric measurements were made in the trabecular region of the metaphysis. The area situated within 1 mm of the epiphysial plate was excluded in order to exclude primary spongiosa [3]. In tibia, 200 μm-thick serial cross-sections were cut from the distal tibiofibular junction to the proximal using a Crystal Cutter (Maruto Instrument Co., Tokyo, Japan). The sections were ground to 30 μm thickness, using Speed Lap (Maruto Instrument Co., Tokyo, Japan). Histomorphometric parameters were measured for the trabecular envelope of the vertebra and the periosteal and endocortical envelopes of the tibia using a semiautomatic image analyzer system (System Supply Co., Nagano, Japan) at 160x magnification [4–7]. Statistical analyses were performed by both nonpaired t tests and one-way analysis of variance (ANOVA).

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Table 1. Bone mineral density of the sixth vertebra and right femur (g/cm²) (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Vertebra (n = 4)</th>
<th>Vehicle (n = 5)</th>
<th>50  (n = 5)</th>
<th>150 (n = 5)</th>
<th>450 µg/kg (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.282 ± 0.004</td>
<td>0.270 ± 0.010</td>
<td>0.245 ± 0.001</td>
<td>0.239 ± 0.003</td>
<td>0.232 ± 0.011</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.248 ± 0.001</td>
<td>0.251 ± 0.004</td>
<td>0.255 ± 0.011</td>
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<tr>
<td>(n = 5)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.279 ± 0.012</td>
<td>0.272 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical difference was observed between treated groups and nontreated groups

*P < 0.05 (vs. control), ^P < 0.05 (vs. vehicle)

Results

Body Weight

No significant differences were detected among the groups in body weight during the whole experimental period.

BMD

No significant differences between the control and vehicle-treated groups were found. However, there were significant differences between G-CSF-treated groups and control, as well as vehicle (P < 0.05) in both the vertebra and the femur. BMD of the vertebra appeared to decrease in a dose-dependent manner after G-CSF treatment (Table 1).

Bone Histomorphometry

Vertebra. An increase of granulocytes in the bone marrow was observed in the G-CSF-treated groups, and trabecular surfaces were occupied by granulocytes as shown in Figure 1. A significant decrease was observed in bone volume (BV/TV) and trabecular thickness (Tb.Th) in the G-CSF-treated groups when compared with both control and vehicle-treated rats (P < 0.05). OS/BS, sLS/BS, dLS/BS, and LS/BS (osteoid surface, single-labeled surface, double-labeled surface, and labeled surface, respectively) were decreased dose dependently. However, there was no significant difference in Tb.N, Tb.S, and MAR among the groups. Both number of osteoclasts (N.Oc/BS) and osteoclast surface (Oc.S/BS) were significantly higher than the low and medium dose G-CSF-treated groups (P < 0.01, respectively) (Table 2).

Tibia. sLS/BS, dLS/BS, LS/BS, and MAR at the endosteal surface decreased significantly in the G-CSF-treated groups (Table 3). However, there were no significant differences in these endpoints at the periosteal surface (Table 4).

Ca, Pi, Alk-p, PGE, Osteocalcin. No significant differences in serum Ca, Pi, and PGE were detected among the groups. Alk-p increased in a dose-dependent manner with increasing G-CSF (Table 5).

Discussion

Though there are many reports regarding the effects of M-CSF or GM-CSF on bone, only a few describe the bone effects of G-CSF. Shinar et al. [8] reported that G-CSF had no effect on the generation of osteoclast-like cells in mouse bone marrow culture. Hosoi et al. [9] reported that G-CSF might have direct stimulatory actions on osteoblast-like cells. No reports of the in vivo effects of G-CSF on bone exist. In the present study, lumbar vertebral body BV/TV and Tb.Th were significantly lower in the G-CSF-treated groups compared with both control and vehicle groups. Similarly, BMD of the vertebra and femur were significantly less in the G-CSF-treated groups. These results suggest that bone formation was inhibited and that the decrease of BMD was partly attributable to an inhibition of the bone formation. As to bone resorption, both osteoclast number and osteoclast surface showed a significant increase with G-CSF treatment.