Effect of Incadronate on Corticosteroid-induced Osteopenia in Rats

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The effect of incadronate, a third-generation bisphosphonate, was evaluated in rats with corticosteroid-induced osteopenia. Male Wistar rats were treated with methylprednisolone acetate (1 mg/kg, s.c.) once daily, 3 days a week for 12 weeks. Other groups received simultaneous treatment with methylprednisolone acetate and incadronate (0.03, 0.3 or 3 mg/kg, p.o.); incadronate was given once daily, 6 days a week for 12 weeks. Bone mineral densities (BMDs) of the second lumbar (L2) vertebra as well as the ultimate compressive strength of the fifth lumbar (L5) vertebra decreased. Incadronate dose-dependently inhibited the loss of L2 BMDs and the decrease in strength of the L5 vertebrae. These results suggest that incadronate may be effective in treating osteopenia accompanying corticosteroid therapy.

Key words—biphosphonate; steroid; osteopenia; bone mineral density; bone strength

INTRODUCTION

Corticosteroids are widely used to treat asthma, rheumatoid arthritis and many other inflammatory diseases due to their potent anti-inflammatory and immunosuppressive effects. It has been known for some time, however, that corticosteroids decrease bone formation by inhibiting osteoblast functions, and that long-term corticosteroid treatment induces bone loss, which may lead to osteoporosis or pathologic fracture. Bisphosphonates show clinical effectiveness in treating metabolic bone disorders such as Paget’s disease, malignancy-associated hypercalce mia, tumor bone metastasis and osteoporosis. Incadronate, a third-generation bisphosphonate, inhibits bone resorption more potently than etidronate, pamidronate or alendronate but does not cause calcification disorders. Incadronate prevents bone mineral density loss, furthermore, maintains the mechanical properties of bones in ovariectomized rats and dogs. Its efficacy in treating corticosteroid-induced osteopenia was not been examined, however, rats with a corticosteroid-induced osteopenia model caused by treatment with methylprednisolone acetate were therefore used to evaluate the efficacy of incadronate in maintaining bone mineral density and mechanical properties.

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MATERIALS AND METHODS

1. Experimental Animals Male Sprague-Dawley rats (aged 13 weeks, weight 410–470 g) purchased from Charles River Japan Ltd. (Kanagawa) were used. All the rats were housed in group cages at a temperature of 23 ± 3°C and a relative humidity of 55 ± 15%. With a 13 h: 11 h light: dark cycle. The rats were fed standard rat chow containing 1.14% calcium and 1.06% phosphorous (CE-2; Japan Clea Co., Ltd., Tokyo) and were given water ad libitum.

2. Drugs Incadronate (lot No. K1759514) was synthesized by Yamanouchi Pharmaceutical Co., Ltd. Methylprednisolone acetate (Depo-Medrol) was purchased from Upjohn Japan Pharmaceutical Co., Ltd. (Tokyo). Incadronate (0.03, 0.3 and 3 mg/kg) was dissolved and diluted with distilled water to a volume of 5 ml/kg body weight. Methylprednisolone acetate (1 mg/kg) was suspended and diluted with saline containing 0.5% methylcellulose to a volume of 2 ml/kg.

3. Experimental Design Sixty rats were divided into 6 groups comprising 10 animals per group. On the day of the start of dosing, the rats in 1 of the 6 groups were sacrificed as a pre-dosing group to provide pre-dosing data. The remaining 5 groups were treated for 12 weeks with one of the following: 1) saline containing 0.5% methylcellulose + distilled water (control group); 2) methylprednisolone acetate + distilled water (vehicle group); 3) methylprednisolone...
acetate+incadronate 0.03 mg/kg (0.03 mg/kg incadronate group); 4) methylprednisolone acetate+incadronate 0.3 mg/kg (0.3 mg/kg incadronate group); or 5) methylprednisolone acetate+incadronate 3 mg/kg (3 mg/kg incadronate group) for 12 weeks. Saline and methylprednisolone were administered subcutaneously once daily 3 times a week for 12 weeks. Distilled water and incadronate were given orally once daily 6 times a week for 12 weeks.

4. Specimen Collection  On the day after the final dosing, all the rats were weighed and anesthetized with ether, and killed by blood letting from the abdominal aorta. The first to the sixth lumbar (L1-6) vertebrae were removed and separated into L1-3, L4, L5 and L6 vertebrae. The L1-3 vertebrae were immersed in 70% ethanol after they were trimmed of the surrounding soft tissues. The L4, L5 and L6 vertebrae were wrapped separately in saline-soaked gauze without trimming of the surrounding soft tissues and stored at −30°C.

5. Measurements

Bone Mineral Density of the L2 Vertebrae  The L2 vertebrae were separated from the L1-3 vertebrae and trimmed of any remaining surrounding soft tissue. The vertebrae were placed, spinous process up, in individual polystyrene containers filled with 70% ethanol to a depth of 2 cm. The bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (QDR2000; Hologic Inc.; Waltham, MA, USA).

Mechanical Strength of the L5 Vertebral Body  Frozen L5 vertebrae were thawed at room temperature, and the remaining surrounding soft tissue was removed. All processes were removed with a diamond cutting disc. The vertebral body was then cut with a diamond disc such that the cut ends were parallel approximately 4.0 mm apart. After the anteroposterior length of the vertebral body was measured, the samples were placed centrally on the smooth surface of a steel disk (2.5 cm diameter) attached to a bone strength tester (TK-252C; Muromachi Kikai Co., Ltd.; Tokyo), and the craniocaudal compression load was applied to the specimen with a steel disk (1.5 cm diameter) at a nominal deformation rate of 2.5 mm/ min. The ultimate compressive strength (N) and structural stiffness (N/mm) were obtained from the load-deformation curve.

6. Statistical Analysis  The measured values are expressed as the mean±the standard error. Pre- and post-treatment comparisons were made in the pre-dosing and control groups, and the effect of methylprednisolone was evaluated by comparing the control and vehicle groups. Student’s t-test was used for these comparisons between two groups. The effect of incadronate was evaluated by Dunnett’s multiple comparison test using the vehicle group as the control.

RESULTS

1. General Condition of the Animals  One animal died during the dosing period in each of the incadronate 0.03 and 0.3 mg/kg groups. No death occurred, however in the incadronate 3 mg/kg group. The absence of dose-dependence suggests that the deaths were caused not by the drug but by an error during administration. At necropsy, the body weight of the control group (631±14 g) was significantly higher than that of the pre-dosing group (466±6 g). Administration of methylprednisolone significantly inhibited weight gain as compared with the control group. There was no significant difference in body weight at necropsy, however, between methylprednisolone-treated animals receiving either vehicle (454±7 g) or incadronate (0.03 mg/kg; 466±6 g, 0.3 mg/kg; 472±8 g, and 3 mg/kg: 440±9 g, respectively).

2. Bone Mineral Density  The L2 vertebral BMD in the control group (0.253±0.004 g/cm²) was significantly higher than that in the pre-dosing group (0.215±0.002 g/cm²). Administration of methylprednisolone for 12 weeks significantly reduced L2 vertebral bone mineral density compared with the control group. Incadronate resulted in a dose-dependent inhibition of BMD loss in the L2 vertebra, with a significant effect observed at doses of 0.3 mg/kg or higher (Table 1).  

3. Mechanical Strength of the L5 Vertebrae  The ultimate compressive strength of the L5 vertebra was significantly higher in the control group (533±24 N) than in the pre-dosing group (374±11 N). Administration of methylprednisolone for 12 weeks significantly reduced the ultimate compressive strength of the L5 vertebra compared with the control group. Incadronate inhibited this decrease dose-dependently, with a significant effect observed at a dose of 3 mg/kg. Structural stiffness did not differ significantly among the groups (Table 2).
Table 1. Effect of Incadronate on Rat Second Lumbar Vertebrae Bone Mineral Density

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Methylprednisolone</th>
<th>Vehicle</th>
<th>Incadronate (mg/kg p.o.)</th>
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<tr>
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<td>BMD (g/cm²)</td>
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<td></td>
<td>0.253 ± 0.004</td>
<td>0.238 ± 0.003**</td>
<td>0.243 ± 0.004</td>
<td>0.250 ± 0.002*</td>
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<td>0.263 ± 0.003**</td>
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</table>

The values in the table show the mean ± standard error for 9-10 animals. ** indicates a significance compared with the control group (p < 0.01; Student’s t-test). * and ** indicate significant differences compared with the vehicle group (p < 0.05, ** p < 0.01; Dunnnett’s multiple comparison test).

Table 2. Effect of Incadronate on Bone Strength of Rat Fifth Lumbar Vertebrae

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Methylprednisolone</th>
<th>Vehicle</th>
<th>Incadronate (mg/kg p.o.)</th>
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<td>Ultimate strength (N)</td>
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<td></td>
<td>533 ± 24</td>
<td>442 ± 23*</td>
<td>427 ± 41</td>
<td>484 ± 38</td>
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<tr>
<td>Structural stiffness (N/mm)</td>
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<td>1571 ± 174</td>
<td>1573 ± 158</td>
<td>1651 ± 184</td>
<td>1580 ± 149</td>
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<tr>
<td></td>
<td>1580 ± 149</td>
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<td>2005 ± 128</td>
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</table>

The values in the table show the mean ± standard error for 7-9 animals. * indicates a significant difference compared with the control group (p < 0.05; Student’s t-test). * indicates a significant difference compared with the vehicle group (* p < 0.05, ** p < 0.01; Dunnett’s multiple comparison test).

DISCUSSION

Corticosteroids are used to treat many disorders because of their potent anti-inflammatory and immunosuppressive effects. Secondary osteoporosis commonly occurs in patients treated with steroids at high doses or for long periods, however, presenting a major clinical problem for steroid treatment. This corticosteroid-induced osteoporosis is thought to result from decreased bone mineral content caused by increased bone resorption and reduced bone formation. Bone loss is due to two factor: a decline in osteoblast functions caused by the direct action of corticosteroids, which inhibit osteoblast differentiation and proliferation, and increased bone resorption associated with secondary hyperparathyroidism, which caused by increased PTH secretion due to a negative calcium balance resulting from inhibition of calcium absorption from the intestine and reduced calcium resorption in the uriniferous tubules. The results of in vivo studies in animals do not show these effects clearly, however. In rats, both positive and negative effects on bone mineral balance, mechanical properties, and morphology have been reported. Comparisons of these and related results are complicated by differences in rat strain, sex, age, diet, steroid formulation, dose level and rate, and route or duration of administration. Nakamuta et al. have shown that treatment with 1 mg/kg of methylprednisolone three times a week for two weeks decreased the levels of metabolic markers associated with bone formation and resorption without adversely affecting the general condition.

The duration of administration was, however, insufficient to induce bone loss. In this study, therefore, treatment with 1 mg/kg of methylprednisolone three times a week for 12 weeks was selected as an appropriate dose to induce osteopenia. Although administration of methylprednisolone at a dose of 1 mg/kg inhibited weight gain, no weight loss or morbid outcome was observed. Furthermore, the L2 vertebral BMD and L5 vertebral ultimate compressive strength were higher in the vehicle group, moreover, than in the pre-dosing group. These results suggest that these reduction of BMD and compressive strength did not result from growth inhibition due to methylprednisolone.

The loss of bone mass that occurs in corticosteroid-induced osteoporosis is PTH-dependent, and the acceleration of bone resorption is thought to be caused by excess PTH secretion due to hyperparathyroidism. In the present study, the vehicle group given methylprednisolone at a dose of 1 mg/kg had a higher serum PTH level (22.7 pg/ml vs 15.7 pg/ml) and significantly lower L2 vertebral BMD than the
control group, suggesting that administration of methylprednisolone at a dose of 1 mg/kg induced osteoporosis by causing mild secondary hyperparathyroidism.

Incadronate significantly inhibited the reduction of L2 vertebral BMD. Methylprednisolone also significantly reduced the ultimate compressive strength of the L5 vertebra. This partially explains why long-term corticosteroid use leads to increased susceptibility to vertebral compression fractures accompanying osteoporosis. Incadronate also dose-dependently inhibits the reduction in ultimate compressive strength caused by methylprednisolone. Corticosteroids have a more pronounced effect on trabecular bone than on cortical bone due to the higher turnover rate of the former.16 Incadronate probably increased the BMD and bone strength of the lumbar vertebrae, therefore, by inhibiting the loss of trabecular bone mass caused by administration of corticosteroids.

In summary, the results show that incadronate inhibited reduction in BMD and bone strength in rats with steroid-induced osteopenia, suggesting that incadronate has promise as an effective therapeutic drug for treating corticosteroid-induced osteoporosis.

REFERENCES