In veterinary medicine, glucocorticoids are one of the most commonly used classes of drugs for treatment of various inflammatory and autoimmune diseases because of their anti-inflammatory and immunosuppressive properties.1 Prednisolone is a synthetic glucocorticoid associated with several adverse effects such as hepatopathy, iatrogenic hyperadrenocorticism, renal disease, and osteopenia.2–5 Prednisolone induces osteopenia by increasing bone resorption and decreasing bone formation.4 There is growing concern about the incidence of osteopenia in dogs because of increases in lifespan. Therefore, osteopenia and osteoporosis induced by hyperadrenocorticism or exogenous prednisolone administration should be prevented by appropriate medical treatment and monitoring BMD.

Although quantitative CT and dual-energy x-ray absorptiometry are the most commonly used techniques for BMD evaluation in humans, their application has been limited to a few cases in veterinary practice. Quantitative CT selectively measures the BMD of trabecular bone, which is more metabolically active than cortical bone, and compared with dual-energy x-ray absorptiometry, more accurately measures BMD by eliminating superimposition with adjacent tissue.3,6 Many medications, including calcitriol, vitamin D, calcium, and bisphosphonates, have been used to prevent or reduce osteopenia in humans.7 Among those medications, bisphosphonates are well-established agents for treating a variety of bone diseases and are most commonly prescribed to treat osteoporosis in humans.7

Alendronate sodium (4-amino-1-hydroxybutyli-dene bisphosphonate sodium salt) is a potent amino bisphosphonate. This drug binds to hydroxyapatite on bone resorption surfaces and inhibits osteoclast-mediated bone resorption.8 In humans, alendronate is used to treat several osteoclastic bone resorption diseases, including osteoporosis, malignant hypercalcemia, osteolytic metastatic bone disease, and Paget's disease and is very effective in preventing glucocortico-
Osteopenia in dogs.

would induce bone loss and that administration of low-dosage prednisolone treatment would induce bone loss and whether administration of alendronate prevents glucocorticoid-induced osteopenia in dogs by measuring trabecular BMD with quantitative CT. We hypothesized that low-dosage prednisolone treatment would induce bone loss and that administration of alendronate would prevent glucocorticoid-induced osteopenia in dogs.

Materials and Methods

ANIMALS

Eight 2- to 3-year-old male Beagles weighing 9 to 13 kg were used in the drug treatment groups; 1 additional clinically normal Beagle was used in the histologic analysis. The study was approved by the Institutional Animal Care and Use Committee of Chonnam National University, Korea, and the animals were cared for in accordance with the Guidelines for Animal Experiments of Chonnam National University. The dogs were housed in individual kennels and were provided commercial dry food and tap water ad libitum. All dogs were healthy, as determined from results of a physical examination, CBC, serum biochemical analysis (activities of alkaline phosphatase, alanine aminotransferase, and amylase and concentrations of albumin, calcium, cholesterol, creatinine, glucose, phosphate, total bilirubin, total protein, and BUN), electrolyte analysis (sodium, potassium, and chloride concentrations), urinalysis, radiography, and abdominal ultrasonography. Serum concentrations of hormones, including thyroxine, free thyroxine, and basal cortisol, were within reference ranges in all dogs.

After CT examination to measure baseline BMD (day 0), each dog was allocated to receive 1 of 2 treatments: prednisolone alone (n = 4) or prednisolone and alendronate (4). In the prednisolone treatment group, prednisolone was administered orally at a dosage of 2 mg/kg, once daily for 2 weeks, 1 mg/kg once daily for 4 weeks, and 0.5 mg/kg once daily for 3 weeks. In the prednisolone-alendronate treatment group, alendronate sodium (2 mg/kg, PO, q 24 h) was orally administered for 9 weeks in addition to the same dosage of prednisolone used in the prednisolone treatment group. In this treatment group, the prednisolone and alendronate were administered at the same time. All drugs in both groups were administered separately from meals to prevent decreased bioavailability. Treatments began immediately after the baseline CT examination on day 0. All CT examinations were performed on days 21, 42, 63, and 150 after start of treatment.

QUANTITATIVE CT EXAMINATION

Food was withheld from each dog for at least 12 hours prior to CT examination. After anesthesia with a combination of zolazepam hydrochloride-tiletamine hydrochloride (1.5 mg/kg, IM) and medetomidine hydrochloride (0.03 mg/kg, IM), CT images of the lumbar vertebrae were acquired by use of a 16-channel multidetector CT scanner. To exclude the effect of table height on BMD, table height was fixed at 125 mm for all procedures. A wedge-shaped quantitative CT phantom containing 2 portions—a water phantom and a bone phantom—was located beneath the lumbar vertebrae for reference. A lateral topogram covering the area from the T12 vertebra to the sacrum was obtained to define appropriate vertebral levels and to determine the angles for subsequent CT scans. With the dog in dorsal recumbency, CT images were acquired for the L2, L3, and L4 vertebrae in 2 ways. First, axial CT images were acquired with a collimator width of 9.6 mm at 130 kVp and 100 mAs, with the transverse section of each vertebral body parallel to the cranial and caudal endplates with or without a tilted gantry. Then, the CT images were reconstructed into 9.6-mm slice thicknesses with a standard bone reconstruction algorithm on a 120-mm field of view, respectively. Then, the lumbar vertebrae were scanned with the installed quantitative CT program with 80 kVp, 24 mAs, and slice thickness of 10 mm on a 108- to 109-mm field of view in sequence for the automatic BMD software. The position of the CT image was at the center of the vertebral body, and the angle of each CT image was corrected parallel to each endplate with or without a tilted gantry. All scan protocols were performed separately by 2 experimenters (SP and JO).

IMAGE ANALYSIS

All CT images were evaluated at a window width of 1,500 HU and a window level of 450 HU at a workstation by 2 independent reviewers (SP and JO). Lumbar trabecular BMD was measured in 2 ways. Manual BMD measurements were performed on axial CT images (slice thickness, 9.6 mm). The trabecular radiodensity was measured by circular and tracer ROIs in each lumbar vertebra. The largest circular ROI was drawn with care not to include the cortical bone and basivertebral veins, and the tracer ROI was drawn including the entire trabecular bone except the pedicle. Each ROI was drawn every time by each operator separately. Phantom density was measured on the same image, and the measured trabecular radiodensity was converted to BMD by use of the following equation:

$$BMD = \frac{200 \text{ HU}}{\text{HU}_{\text{ph}} - \text{HU}_{\text{w}}}$$

Where HU is the measured trabecular radiodensity, HU ph is the radiodensity of the bone phantom (containing 200 mg of calcium hydroxyapatite/cm²), and
Region of interest was automatically drawn to the trabecular bone in the automatic BMD software, based on vertebral landmarks such as vertebral canal, ventral point of vertebral body, bilateral intermediate points of the vertebral body, ventral point of the vertebral canal, and the bone and water portion of the phantom for reference. Each reviewer intervened to reposition the landmark for the appropriate ROI setting when the ROI was created in an incorrect position.

**HISTOLOGIC EXAMINATION**

One dog each from the prednisolone and the prednisolone-alendronate treatment groups was euthanized by IV injection of potassium chloride during anesthesia after the CT examination on day 63 for histologic examination of bone structure. A clinically normal research dog was also similarly euthanized for evaluation in this and another research project. The lumbar vertebrae were decalcified in 10% nitric acid solution for > 2 weeks. The tissues were then neutralized in 5% sodium sulfate for 12 hours before dehydration in an ethanol-xylene series. Then, bone tissue slices were stained with H&E stain. The percentage area of the trabecular bone, marrow fat, and erythropoietic marrow was measured in each histologic slice.

**STATISTICAL ANALYSIS**

Statistical analysis was performed with the aid of a statistical program. Analysis of reproducibility between image reviewers was performed by means of an ICC test. Repeated-measures ANOVA was used to investigate significance of the BMD variations in regard to days after the start of treatment and the BMD differences between the prednisolone treatment group and the prednisolone-alendronate treatment group. It was also used to compare the BMD differences among measured vertebral levels (the L2, L3, and L4 vertebrae) and the automatic, circular ROI, and tracer ROI methods. The level of significance was set at a value of P < 0.05.

**Results**

Interoperator reproducibility was high for the automatic software (ICC, 0.544 to 0.999; mean, 0.967), except on the CT images for the L2 vertebra (ICC, 0.544). The tracer ROI (ICC, 0.804 to 0.997; mean, 0.955) had higher values than those for circular ROI (ICC, 0.704 to 0.995; mean, 0.924; Figure 1). Bone mineral densities of L2, L3, and L4 vertebrae were determined by use of each of the BMD measuring methods. No significant difference in BMD was evident among days by use of the circular ROI method, tracer ROI method, or automatic software method. Therefore, the tracer ROI method with slice thickness of 9.6 mm and the highest ICC was used for further statistical analysis of lumbar BMD.

Bone mineral density data were available for 8 dogs (4 dogs/treatment group) at days 0, 21, 42, and 63 after start of treatment; data were available for 6 dogs (3 dogs/treatment group) at day 150. Among the 8 dogs, mean baseline BMD was 297.63 mg/cm$^3$ (range, 288.69 to 303.44 mg/cm$^3$). After the baseline CT examination, the 8 dogs were allocated to the prednisolone and prednisolone-alendronate treatment groups (4 dogs/group); there was no significant difference in mean BMD between the prednisolone (295.27 cm$^3$) and prednisolone-alendronate treatment (299.99 cm$^3$) groups. The BMDs for the
L2, L3, and L4 vertebrae decreased significantly \((P < 0.05)\) after drug administration in both groups, compared with the corresponding baseline BMD. In the prednisolone treatment group at day 42, the mean maximal decrease in lumbar BMD was 84.7\% (range, 83.8\% to 85.4\%) of the baseline value (Table 1). Mean BMD increased to 87.9\% (range, 87.6\% to 88.1\%) of the baseline value at day 63 and recovered to 91.6\% (range, 91.5\% to 91.9\%) of the baseline value at day 150. In the prednisolone-alendronate treatment group at day 21, the mean maximal decrease in lumbar BMD was 91.0\% (range, 88.9\% to 92.6\%) of the baseline value (Table 2). Mean BMD increased to 93.8\% (range, 93.4\% to 94.3\%) of the baseline value at day 63, and then recovered to 96.7\% (range, 93.2\% to 99.0\%) of the baseline value at day 150. Although the mean BMD for each evaluated lumbar vertebra was generally lower on each examination day in the prednisolone treatment group, compared with findings in the prednisolone-alendronate treatment group, no significant difference in BMD was detected between the groups for any lumbar region at any time point. During the period of decreasing BMD, no evidence of pathological fracture was detected in any dog.

On day 63, 1 dog in the prednisolone treatment group and 1 dog in the prednisolone-alendronate treatment group were euthanized to perform histologic assessment of bone tissue (Figure 2). A clinically normal Beagle was also euthanized for tissue assessments. Histologic examination of L3 was performed in each dog. In the vertebral marrow cavity of the clinically normal dog, the bone trabecular area was 52\%, marrow fat cell area was 23\%, and erythropoietic marrow area was 25\%. In the vertebral marrow cavity of the prednisolone-treated dog, bone trabecular area was 41\%, marrow fat cell area was 24\%, and erythropoietic marrow area was 35\%. In the vertebral marrow cavity of the prednisolone-alendronate-treated dog, bone trabecular area was 43\%, marrow fat cell area was 25\%, and erythropoietic marrow area was 32\%. By comparing data for the treated dogs with that for the clinically normal dog, the trabecular area ratio was 0.79 (41\% vs 52\%) for the prednisolone-treated dog and 0.85 (43\% vs 52\%) for the prednisolone-alendronate-treated dog. Comparison of CT images of these dogs revealed slight but no significant differences (Figure 3).

Table 1—Mean ± SD BMD (mg/cm³) in the L2, L3, and L4 vertebrae of 4 dogs administered prednisolone (beginning day 0) at a dosage of 2 mg/kg once daily for 2 weeks, 1 mg/kg once daily for 4 weeks, and 0.5 mg/kg once daily for 3 weeks, as determined by use of the tracer ROI method (slice thickness, 9.6 mm).

<table>
<thead>
<tr>
<th>Days after treatment initiation</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (pretreatment baseline)</td>
<td>298.20 ± 30.49</td>
<td>298.92 ± 27.71</td>
<td>288.69 ± 26.21</td>
</tr>
<tr>
<td>21</td>
<td>260.19 ± 44.51</td>
<td>260.00 ± 33.75</td>
<td>251.02 ± 37.59</td>
</tr>
<tr>
<td>42</td>
<td>254.75 ± 41.62</td>
<td>253.42 ± 36.08</td>
<td>242.00 ± 35.92</td>
</tr>
<tr>
<td>63</td>
<td>261.36 ± 29.71</td>
<td>263.22 ± 26.23</td>
<td>253.76 ± 28.00</td>
</tr>
<tr>
<td>150*</td>
<td>270.52 ± 30.66</td>
<td>267.81 ± 21.08</td>
<td>259.53 ± 19.13</td>
</tr>
</tbody>
</table>

For each dog, CT images of the lumbar vertebrae were acquired by use of a 16-channel multidetector CT scanner (table height was fixed at 125 mm for all procedures). Computed tomographic images were acquired from the L2, L3, and L4 vertebrae with the dog in dorsal recumbency in 2 ways. First, axial CT images were acquired with a collimator width of 9.6 mm at 130 kVp and 100 mAs with the transverse section of each vertebral body parallel to the cranial and caudal endplates with or without a tilted gantry. Then, the CT images were reconstructed into 9.6-mm slice thicknesses with a standard bone reconstruction algorithm on a 120-mm field of view. Second, the lumbar vertebrae were scanned with the installed quantitative CT program with 80 kVp, 24 mAs, and slice thickness of 10 mm on a 108- to 109-mm field of view for the automatic BMD software. The position of the CT image was at the center of the vertebral body, and the angle of each CT image was corrected parallel to each endplate with or without a tilted gantry. All CT images were evaluated at a window width of 1,500 HU and a window level of 450 HU at a workstation by 2 independent reviewers. Lumbar trabecular BMD was measured in 2 ways. Manual BMD measurements were performed on axial CT images (slice thickness, 9.6 mm). The trabecular radiodensity was measured by circular ROIs (C9.6 method) and tracer ROIs (T9.6 method) in each lumbar vertebra. *Data are derived from 3 dogs.

Table 2—Mean ± SD BMD (mg/cm³) in the L2, L3, and L4 vertebrae of 4 dogs administered alendronate sodium (2 mg/kg, PO, q 24 h) for 9 weeks, in addition to the same dosage of prednisolone used in the prednisolone treatment group in Table 1, as determined by use of the tracer ROI method (slice thickness, 9.6 mm).

<table>
<thead>
<tr>
<th>Days after treatment initiation</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (pretreatment baseline)</td>
<td>293.91 ± 33.98</td>
<td>303.44 ± 26.89</td>
<td>302.63 ± 42.82</td>
</tr>
<tr>
<td>21</td>
<td>272.04 ± 37.48</td>
<td>277.71 ± 27.90</td>
<td>269.06 ± 42.30</td>
</tr>
<tr>
<td>42</td>
<td>277.09 ± 39.67</td>
<td>284.83 ± 32.70</td>
<td>282.60 ± 44.01</td>
</tr>
<tr>
<td>63</td>
<td>280.66 ± 39.43</td>
<td>287.26 ± 33.81</td>
<td>282.10 ± 38.59</td>
</tr>
<tr>
<td>150*</td>
<td>291.21 ± 31.71</td>
<td>296.93 ± 21.82</td>
<td>281.98 ± 42.27</td>
</tr>
</tbody>
</table>

See Table 1 for key.
These results suggested that trabecular BMD in the prednisolone treatment group was slightly lower than that of the prednisolone-alendronate treatment group.

Discussion

In the present study, the induction of bone loss by a low-dosage regimen of prednisolone and prevention of glucocorticoid-induced osteopenia by administration of alendronate was investigated with quantitative CT in Beagles. Lumbar BMD was measured by use of automatic software and manual methods. Because the BMD software was actually designed for human vertebrae, the ROI created by the automatic software was occasionally placed incorrectly. In such instances, an operator intervened with the ROI setting to obtain correct placement. Nevertheless, the automatic software measurement was faster and simpler than the manual methods. However, the automatic method requires a BMD software program, which is provided as an extra-cost option by most CT manufacturers. The manual method was more complex and time-consuming because bone radiodensity, measured in HU, on CT images had to be converted to BMD by use of an equation, but the process was performed without additional software. The CT images for the manual method were reconstructed into 9.6-mm-thick slices similar to the automatic method with a slice thickness of 10 mm. No significant difference in BMD derived by means of the automatic, circular, and tracer ROI methods was detected. Bone mineral density measured with the tracer ROI method was chosen for further statistical analyses because of high interoperator reproducibility.

Many studies investigating the osteopenic effects of prednisolone have been performed in veterinary medicine as well as in human medicine because prednisolone is commonly administered for its anti-inflammatory and immunosuppressive properties. In a previous study involving dogs, prednisolone-induced bone loss was assessed after oral administration of prednisolone (2 mg/kg) once daily for 4 weeks, and BMD of the lumbar vertebrae decreased significantly about 14% of baseline BMD. In that study, a high dosage of prednisolone was used to alleviate autoimmune disease such as immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, and polyarthritis. Even in those cases, prednisolone was usually tapered to a lower dosage because of adverse effects such as gastroenteritis, glomerulonephritis, and hepatopathy. A low dosage of prednisolone administered...
for a short period is more commonly used to treat pruritus, dermatitis, allergy, and hypersensitivity disorders. Therefore, a lower dosage of prednisolone with dosage tapering was applied in the present study to reflect the more clinical situation.

Across the 3 lumbar vertebrae evaluated, the mean BMD of the prednisolone-treated dogs decreased at day 21 to 87.1% of the baseline BMD; at day 42, the value had decreased to 84.7% of the baseline BMD. Bone mineral densities of the prednisolone treatment group increased at day 63 to 87.9% of the baseline BMD; at day 150 (or approx 3 months after the end of prednisolone administration), the value had recovered to 91.6% of the baseline BMD. That is, lumbar vertebral BMD decreased while 2 mg of prednisolone/kg was being administered to the dogs, then increased with a reduced dosage. The effect of prednisolone on bone loss was temporary and reversible; and BMD of lumbar vertebrae recovered gradually after prednisolone administration ceased.

Alendronate is used in veterinary medicine for treatment of dogs and cats for refractory hypercalcemia, feline odontoclastic resorptive lesions, and osteosarcoma. However, few studies about drug prevention for bone loss induced by endocrine diseases such as hyperadrenocorticism or exocrine prednisolone administration have been performed, to our knowledge. In the present study, lumbar vertebral BMD also significantly decreased, compared with the baseline value, during the early experimental period in the prednisolone-alendronate treatment group. Although the decrease in BMD was detected at day 21 (with improvement thereafter) in this group and was generally less severe than that in the prednisolone treatment group, no significant difference in BMD between groups was detected. Bone mineral densities of the prednisolone-alendronate treatment group also increased at day 42 to 93.8% of the baseline BMD; at days 63 and 150, values were 94.5% and 96.7% of the baseline BMD, respectively. Thus, alendronate had little effect on preventing low-dosage prednisolone-induced osteopenia in the study dogs.

The mean BMD in the prednisolone treatment group decreased temporarily and then recovered gradually after tapering the prednisolone dosage and then stopping the drug administration. The extent of BMD decrease in the prednisolone treatment group was not significantly different than that of the prednisolone-alendronate group. The low-level glucocorticoid-induced bone loss in the present study, compared with that achieved in previous studies, was due to the low dosage and tapering protocol of prednisolone administration.

In the present study, trabecular BMD in lumbar vertebrae was measured by quantitative CT to assess bone loss caused by low-dosage prednisolone administration and evaluate the effectiveness of alendronate for preventing prednisolone-induced bone loss in dogs. A low-dosage regimen of prednisolone caused a significant decrease in BMD during the early treatment period, but the bone loss was temporarily and reversible. No meaningful preventative effect of alendronate against low-dosage prednisolone-induced bone loss was found. These results have suggested that a low dosage of prednisolone can be used in dogs with little concern for development of osteopenia or for the requirement for concomitant administration of medication to prevent bone loss.

Acknowledgments

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Footnotes

b. Fosamax, Merck Sharp & Dohme, Pavia, Italy.
c. Zoletil, Virbac, Carros, France.
d. Domitor, Orion Corp, Espoo, Finland.
e. Siemens Emotion 16; Siemens, Forchheim, Germany.
f. Calcium hydroxyapatite rod (0 and 200 mg/cm 3), Siemens, Forchheim, Germany.
g. Symo Ostego CT, Siemens, Forchheim, Germany.
h. SPSS Statistics, version 20, IBM Corp, Armonk, NY.

References


