1α-Hydroxyvitamin D₃ Prevents the Decrease of Bone Mineral Density in Lactating Beagles

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MATERIALS AND METHODS

Animals: Seven beagles, 2–5 years old, were used in this study, and were kept in individual cages. Room temperature was preserved at between 15°C and 22°C, relative humidity was 45–70%, a ventilation system was employed and natural light was provided. The beagles were kept on normal chow ad libitum. The amount of food fed to each beagle varied by using an oral catheter. Dogs were administered the compound three times in a week for 14 weeks, from 2 weeks after weaning. Drinking water was given ad libitum.

Administration and schedule: Seven beagles were divided into two groups, three dogs were administered 1α(OH)D₃ (Chugai Pharm. Co., Ltd.) at a dose of 0.1 µg/kg and the control group was given vehicle. Medium chain triglyceride was used as vehicle and 1α(OH)D₃ was adjusted to a concentration of 0.5 µg/ml with the vehicle. Administration volume was at a dosage of 0.2 ml/kg, and was administered by using an oral catheter. Dogs were administered the compound three times in a week for 14 weeks, from 2 weeks before delivery until 12 weeks postpartum.

Coefficient of variation of bone mineral density by DXA: Using a model of DCS-3000 (Aloka Co., Ltd.), lumbar vertebral (L2-L4) and tibial bone mineral density (BMD) in beagles were measured under pentobarbital anesthesia. The vertebral BMD was measured in the supine position and tibial BMD was in lateral position. The X-ray applied from the belly side on measurement of vertebral BMD and from left side on that of tibial BMD. Two beagles were selected to determine the coefficient of variation (CV(%)=standard deviation/mean × 100) on BMD by DXA, one had a...
maximum weight and another had a minimum weight in this experiment. Measurement was performed five times to obtain the CV(%) of BMD by DXA on both vertebrae and tibiae.

**Measurement of bone mineral density by DXA:** All data are the average of the two measurements of BMD. Measurements were performed at mating as initial values, at delivery and in every two weeks after delivery.

**Urine and serum sampling:** Blood was collected from intermediate antebrachial vein. The blood was centrifuged and serum was obtained. Urine was collected using a catheter from bladder under ketamine anesthesia. Each sample was obtained at 24 hr after the administration. These samplings were undertaken at mating, delivery and in 2 weeks intervals following delivery.

**Serum and urinary parameter assessment:** Serum and urinary calcium were assayed by OCPC method with calcium C-test kit (Wako Pure Chemicals Industries, Ltd.). Serum alkaline phosphatase (ALP) was measured by GSCC method with ALP kit (Iatron). Tartrate resistant acid phosphatase (TRACP) was determined by Bessey-Lowry method with ACP kit (Nittobo Co., Ltd.). Urinary creatinine was measured by colorimetric method with creatinine kit (Iatron).

**Statistical analysis:** Analysis of the number of neonates was performed by Student’s t-test. Statistical significance on longitudinal data of BMD was analyzed by mixed model with group, time and group-time interaction as fixed, subject as random effect. P-values of less than 0.05 were defined as significant.

**RESULTS**

All beagles were found to suffer from a loss of appetite throughout the pregnancy and post-partum. The number of neonates was not statistically different between experimental groups. Means and standard deviations (SD) of the number were 7.3 ± 1.3 in the control and 6.3 ± 0.48 in the 1α(OH)D₃ group. Mean, SD and CV(%) of vertebral and tibial BMD were shown in Table 1. From these results, we concluded that a time course change of vertebral BMD and tibial BMD could be detected by DXA.

Figure 1 shows the BMD changes in vertebrae in percentage. BMD at mating sets as 100%. It was found that vertebral BMD was at a minimum at 4 weeks after delivery in the two groups. The BMD in the control group and the 1α(OH)D₃ treatment group were reduced to 24% and 12%, respectively. Vertebral BMD in both control and 1α(OH)D₃ groups recovered by 95% and 103%, respectively, after weaning. Treatment of 1α(OH)D₃ prevented the decrease of BMD in lactation and significantly induced the recovery of the reduced BMD after weaning.

**Table 1. Coefficient of variation on vertebral and tibial BMD by DXA in beagles**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Mean ± SD (mg/cm²)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. vertebrae, weight=10.0 kg</td>
<td>750 ± 3.94</td>
<td>0.52</td>
</tr>
<tr>
<td>1. tibia</td>
<td>503 ± 1.48</td>
<td>0.30</td>
</tr>
<tr>
<td>2. vertebrae, weight=8.0 kg</td>
<td>669 ± 3.11</td>
<td>0.47</td>
</tr>
<tr>
<td>2. tibia</td>
<td>409 ± 2.71</td>
<td>0.66</td>
</tr>
</tbody>
</table>

respectively. Vertebral BMD in both control and 1α(OH)D₃ groups recovered by 95% and 103%, respectively, after weaning. Treatment of 1α(OH)D₃ prevented the decrease of BMD in lactation and significantly induced the recovery of the reduced BMD after weaning.

Time course changes of tibial BMD are shown in Fig. 2.
The BMD were also reduced during lactation but the degrees in tibial BMD were smaller than that of the vertebrae. Maximum reduction of the BMD was 8% at 4 weeks in the control and 5% at 6 weeks in the $1\alpha(OH)D_3$ group.

Figure 3 shows the results of serum and urinary biochemical parameters. Alkaline phosphatase (ALP) levels in both the control group and the $1\alpha(OH)D_3$ treated group increased in the breast-feeding period, and these levels returned to the initial levels at mating after weaning in both groups (Fig. 3-A). Tartrate-resistance acid phosphatase (TRACP) levels were unchanged during lactational period and by the treatment of $1\alpha(OH)D_3$ (Fig. 3-B).

Hypercalcemia (Fig. 3-C) and hypercalciuria (Fig. 3-D) were not induced by the treatment of $1\alpha(OH)D_3$.

**DISCUSSION**

Time course changes in vertebral and tibial BMD in maternal beagles from lactation to weaning periods could be detected by DXA. Beagles lost their vertebral BMD as much as 24% compared to the initial level at mating during the 4 weeks breast feeding period, since calcium must be mobilized into the breast milk. These beagles took about 5 g of calcium in a day during the lactation period from diet.
Although, the efficiency of intestinal calcium absorption is unknown, the total amount of calcium intake appears to be sufficient for beagles in lactation.

We observed biochemical bone markers, ALP as bone formation parameter and TRACP as bone resorption parameter. Elevated ALP activities suggested the induction of bone formation during lactation period, while TRACP activities were not found the increment based on lactation. TRACP might be unsuitable for observing bone turn over in lactation. Since treatment of \( 1\alpha(\text{OH})_2\) bound to protein (PTHrP) had no effect on ALP activities, the bone formation might not be affected by administration of \( 1\alpha(\text{OH})_2\). In clinical study, bone resorption was inhibited by the treatment of \( 1\alpha,25(\text{OH})_2\) [1]. On bone formation, ALP and bone gla-protein are up-regulated by in \textit{in vitro} [15, 18], but the effect of \( 1\alpha,25(\text{OH})_2\) in \textit{in vivo} is still unclear. Administration of \( 1\alpha(\text{OH})_2\) at a dose of 0.1 \( \mu \text{g/kg} \) might be adequate dose in lactating beagles, because the preventive effect on BMD was observed without adverse effects. Daily administration of \( 1\alpha(\text{OH})_2\) at a dose of 0.1 to 0.2 \( \mu \text{g/kg} \) for 10 weeks induced hypercalcemia in beagles in safety assessment study (unpublished data). Administration of \( 1\alpha(\text{OH})_2\) three times in a week at the same dose for 14 weeks was seemed to be enough dose to make the calcium balance in the beagles positive.

Factors related to the reduction of bone mineral in lactation are still unknown. Parathyroidectomy or vitamin D deficient rats were used to make the mechanism of the lactational bone loss clear, but PTH and vitamin D were reported not to relate the bone loss [9, 11]. One of the candidates for this bone loss is thought to be parathyroid hormone related peptide (PTHrP). PTHrP is produced extremely in the mammary glands during lactation and PTHrP in maternal blood was found to be higher level than normal [4, 8]. Bone resorption is induced by PTHrP as well as PTH [20], therefore, the bone loss in lactation may be induced by PTHrP. \( 1\alpha(\text{OH})_2\) is believed to be converted into \( 1\alpha,25(\text{OH})_2\) before revealing the biological function in \textit{in vivo}. Therefore, serum \( 1\alpha,25(\text{OH})_2\) level in \( 1\alpha(\text{OH})_2\) treatment group would be elevated. The elevated \( 1\alpha,25(\text{OH})_2\) might suppress PTHrP production in lactation, since PTHrP is negatively regulated by \( 1\alpha,25(\text{OH})_2\) [12], although, this remains to be established.

A greater difference was observed on the decrease of vertebral BMD than on tibial BMD. The vertebrae are rich in trabecular bone, and bone resorption would have mainly occurred in trabecular bone rather than in cortical bone in the period of breast-feeding [6, 16]. Otherwise, weight load would be one of the related factor on bone loss, tibiae would be under more weight load than that in vertebrae in beagles because of pronograde.

From our experiment, we observed that lactation induced bone loss in beagles, long-lived mammals, as well as rats. Further, \( 1\alpha(\text{OH})_2\) prevented bone loss in lactation and induced recovery of decreased bone minerals after weaning.

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REFERENCES


