Effect of Active Vitamin D₃ Analogs on the Osteoporosis of Rats Caused by Ovariectomy

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ABSTRACT. The effects of 1,24(R)-dihydroxyvitamin D₃ (1,24(R)(OH)₂D₃), 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) and 1 α -hydroxyvitamin D₃ (1 α (OH)D₃) on the development of osteoporosis in ovariectomized rats were investigated biochemically and histomorphologically. Vitamin D₃ analogs dissolved in corn oil were given orally to the rats for 6 months after the ovariectomy. The sham-operated control animals received the vehicle alone. Ovariectomy was followed by the development of osteoporosis after 6 months of observation. This was characterized by a reduction in ash content of the tibia and histologically by a disappearance of the trabecular bone in tibial metaphysis. Biochemical studies showed significant decreases in serum calcium and 1,25(OH)₂D concentrations, and significant increases in serum immunoreactive parathyroid hormone (iPTH) level in ovariectomized rats. These findings suggest that ovariectomy partially impairs intestinal absorption of calcium, increases bone resorption, and results in a negative calcium balance, which may contribute to the development of osteoporosis in rats. The administration of 1,24(R)(OH)₂D₃, 1,25(OH)₂D₃ and 1 α (OH)D₃ increased dose-dependently serum and urinary calcium, but decreased serum iPTH levels to the control level and prevented the development of osteoporosis histologically. These results support the view that active vitamin D₃ analogs may be valuable tools for the treatment of osteoporosis.—**KEY WORDS:** osteoporosis, ovariectomy, vitamin D₃

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Although osteoporosis is a relatively widespread disorder among the aged of both sexes of animal and human, its incidence rises markedly in females. Loss of ovarian function and estrogen deficiency in particular can be considered to be the most important factor in the etiology of osteoporosis. In spite of extensive clinical treatment of postmenopausal osteoporosis with estrogen and anabolic hormone, their mechanisms of action and therapeutic usefulness have not been completely established [16]. This situation has arisen partly because of the difficulty in setting up adequately controlled clinical trials in women, and partly because of the lack of an appropriate experimental model for this disease [1].

Our previous study [9] and some authors [7, 10] have suggested that the administration of active vitamin D₃ analogs partly prevented

the development of osteoporosis in rats. It is very important to know the long-term effects of active vitamin D₃ administration on the skeleton and above all, whether or not such therapy can prevent the development of osteoporosis after ovariectomy in rats. The purpose of the present study was to investigate the nature and pathogenesis of osteoporosis in ovariectomized rats and to evaluate the effects of active vitamin D₃ analogs on this model.

MATERIALS AND METHODS

Eighty female rats of the Sprague-Dawley strain at 6 weeks of age were used. Seventy of them were ovariectomized and the remaining ten were sham-operated. The latter intact control group was called group I. The operations were performed under pentobarbital an-

esthesia. The day after the operation, the ovariectomized animals were divided into seven groups of ten animals each as follows: group II, ovariectomized controls; group III, administered $0.02 \mu\text{g/kg}$ of $1,24(\text{R})(\text{OH})_2\text{D}_3$; group IV, $0.10 \mu\text{g/kg}$ of $1,24(\text{R})(\text{OH})_2\text{D}_3$; group V, $0.02 \mu\text{g/kg}$ of $1,25(\text{OH})_2\text{D}_3$; group VI, $0.10 \mu\text{g/kg}$ of $1,25(\text{OH})_2\text{D}_3$; group VII, $0.02 \mu\text{g/kg}$ of $1\alpha(\text{OH})\text{D}_3$; group VIII, $0.10 \mu\text{g/kg}$ of $1\alpha(\text{OH})\text{D}_3$. Vitamin D_3 analogs, synthesized by Teijin Ltd. and dissolved in corn oil, were given orally to the rats for 6 months ($0.10 \text{ ml}/100 \text{ g}$). The intact and ovariectomized control groups received the vehicle alone. All the rats were fed ad libitum with a standard laboratory diet (CREA Japan, Inc.) containing 24% protein, 4.4% fat, 1% calcium and 1% phosphorus with 2,000 units of vitamin D per kg throughout the experiment. During the course of the experiment, clinical symptoms, body weights and food consumption were recorded at regular intervals. Urinary calcium (Ca) and inorganic phosphorus (Pi) were measured 2nd, 4th and 6th months after the beginning of vitamin D_3 treatment. After 6 months of ovariectomy and treatment, blood was drawn from the carotid artery under pentobarbital anesthesia. Serum total protein, albumin, Ca and Pi concentrations, and alkaline phosphatase (Alp) activity were determined. Serum and urinary biochemical parameters were measured using an autoanalyzer (HITACHI 706D). Serum concentration of 25-hydroxyvitamin D ($25(\text{OH})\text{D}$), 24,25-dihydroxyvitamin D ($24,25(\text{OH})_2\text{D}$) and 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) was measured. A competitive protein binding assay [18] was utilized for determination of $25(\text{OH})\text{D}$ and $24,25(\text{OH})_2\text{D}$ and radio receptor assay [5] for $1,25(\text{OH})_2\text{D}$. Serum iPTH concentration was assayed using anti-bovine PTH antisera specific for the N-terminal fragment (BioScience laboratory in California).

The right and left femurs and tibias were dissected free and examined as follows;

Femoral morphology; The X-ray of the femur was examined under magnification, and the total length was measured. The cortical thickness of the femur was measured at the middle of the whole bone and expressed as a percentage of the total width of the bone [13]. *Bone composition*; The left tibia was dried in an oven at 110°C for 5 days for measurement of dry weight and was then defatted in a methanol: trichloroethylene (1:1) solution for 3 days. Defatted dried bones were weighed and ashed in porcelain crucibles at 900°C for 5 hours to obtain the ash weight.

Bone histology; some right tibial bones were fixed in 70% ethanol and embedded without decalcification in polyester resin (Ligorac®). Mid-frontal longitudinal sections ($50\text{--}60 \mu$) were cut from the proximal end of the tibia and serial cross sections were cut from the metaphysis of the bone. Then the sections underwent histological examination using contact microradiography (CMR). CMRs of tibial cross sections obtained from each group were magnified and projected on graph paper, and trabecular bone thickness was measured at cross-points of the trabeculae and 5-mm-spaced intersecting lines.

Mineral apposition rate: The tibial cortical bones were investigated by the tetracycline double labeling method. After 6 months, each rat was injected intraperitoneally, twice at 10-day intervals with oxytetracycline (Terramycin, Pfizer) in a dose of 15 mg/kg of body weight. Three serial cross sections were cut from the tibial metaphysis of undecalcified specimens made by the same method as described above. The mineral apposition rate was obtained by measuring the width of accreted tetracycline double labels under a fluorescent microscope.

Differences between means were determined by Student's t-test.

RESULTS

During the experimental period, no abnor-

Table 1. Serum biochemical findings in ovariectomized rats orally treated with active vitamin D₃ analogs

Group	Dose (μg/kg)	T. protein (g/dl)	albumin (g/dl)	Alp (K-AU)	Calcium (mg/dl)	Inorganic phosphorus (mg/dl)
Intact control	—	6.4±0.2 ^{a)***b)}	2.95±0.10**	5.9±0.7	10.2±0.1***	4.6±0.6
Ovx-control	—	5.4±0.1	2.35±0.03	5.9±0.3	9.1±0.1	4.4±0.3
1,24(R)(OH) ₂ D ₃	0.02	5.4±0.1	2.28±0.03	6.6±1.2	10.2±0.1***	5.6±0.2*
	0.10	5.4±0.1	2.25±0.02*	5.1±0.7	10.2±0.2***	6.3±0.5**
1,25(OH) ₂ D ₃	0.02	5.6±0.2	2.30±0.04	5.1±1.0	9.6±0.1	5.1±0.3
	0.10	5.8±0.1**	2.32±0.03	6.8±0.5	10.2±0.1***	6.2±0.3**
1α(OH)D ₃	0.02	5.8±0.1*	2.37±0.04	4.5±0.6	9.5±0.1	4.6±0.4
	0.10	5.4±0.1	2.38±0.03	5.7±0.4	10.5±0.2***	6.3±0.4**

a) Mean±SE (n=6).

b) *p<0.05, **p<0.01, ***p<0.001 vs Ovx-control group.

Table 2. Urinary calcium and phosphorus excretions in ovariectomized rats treated with active vitamin D₃ analogs

Group	Dose (μg/kg)	Ca (mg/day)			Pi (mg/day)		
		2 months	4 months	6 months	2 months	4 months	6 months
Intact control	—	0.53±0.30 ^{a)}	1.28±0.43	1.25±0.34	19.7±1.8	17.8±0.9*	13.3±1.3
Ovx-control	—	0.21±0.06	0.54±0.08	0.82±0.15	22.7±1.9	12.7±2.1	13.7±0.7
1,24(R)(OH) ₂ D ₃	0.02	0.61±0.21	4.79±0.50***	4.72±0.49***	19.8±1.2	24.4±0.9***	19.0±1.7*
	0.10	2.91±0.34*** ^{b)}	7.14±0.38***	9.38±0.95***	24.5±0.8	21.7±2.4*	21.6±1.5**
1,25(OH) ₂ D ₃	0.02	1.43±0.46*	4.31±0.85**	6.92±0.72***	25.2±2.9	19.8±1.6*	22.6±3.0*
	0.10	3.97±0.59***	6.67±0.51***	8.64±2.18**	24.8±2.1	25.9±2.0**	18.6±4.1
1α(OH)D ₃	0.02	0.38±0.07	2.94±0.60**	3.35±0.48***	21.0±1.0	21.7±1.5**	18.9±1.1**
	0.10	3.99±0.93**	7.96±0.41***	8.68±0.72***	26.9±0.9	25.9±2.6**	22.4±2.5**

a) Mean±SE (n=5).

b) *p<0.05, **p<0.01, ***p<0.001 vs Ovx-control group.

mal symptom was observed in all animals. Intact and ovariectomized (ovx) rats had similar body weights at the start of this study and they increased as the study progressed. At the end of experiment, the mean body weight of the ovx-rats was significantly higher than the weight of the corresponding sham-operated intact control. The increase in food intake was furthermore observed after ovariectomy in rats. No influence of any vitamin D₃ analog on body weight and food consumption was observed at both low and high dose groups.

Serum biochemical measurements are given in Table 1, and urinary findings in Table 2. Serum total protein and albumin concentra-

tions in ovariectomized rats (ovx-control) were significantly lower than those in sham-operated intact rats (intact control), and these lowered values appeared to be not affected by the doses of active vitamin D₃ analogs. Ovariectomy also decreased the serum Ca levels when compared with intact rats. At low dose (0.02 μg/kg) administration, 1,24(R)(OH)₂D₃ significantly elevated the serum Ca to the physiological normal range, whereas 1,25(OH)₂D₃ and 1α(OH)D₃ tended to increase the serum Ca but not significantly when compared with the ovx-control. In the groups treated with the high dose (0.10 μg/kg) of these three vitamin D₃ analogs, serum Ca levels increased significantly as a result of

Table 3. Effect of active vitamin D₃ analogs on serum immunoreactive parathyroid hormone and vitamin D metabolites in ovariectomized rats

Group	Dose ($\mu\text{g/kg}$)	iPTH (pg·eq/ml)	25(OH)D (ng/ml)	1,25(OH) ₂ D (pg/ml)	24,25(OH) ₂ D (ng/ml)
Intact control	—	437 ± 42 ^a ***b)	33.4 ± 3.8	125.5 ± 12.5**	4.54 ± 0.64
Ovx-control	—	975 ± 146	26.8 ± 6.7	66.8 ± 7.1	4.02 ± 1.00
1,24(R)(OH) ₂ D ₃	0.02	452 ± 22**	11.0 ± 2.0***	49.0 ± 10.0	1.98 ± 0.34**
	0.10	555 ± 108	14.8 ± 2.9**	54.8 ± 18.1	1.81 ± 0.28**
1,25(OH) ₂ D ₃	0.02	565 ± 114	17.9 ± 2.8***	66.0 ± 17.1	6.64 ± 1.12
	0.10	482 ± 97*	12.6 ± 3.1*	97.3 ± 24.1	8.56 ± 1.33**
1 α (OH)D ₃	0.02	595 ± 143	11.9 ± 1.3**	116.0 ± 22.0	6.28 ± 1.42
	0.10	532 ± 52*	20.5 ± 2.1**	135.0 ± 16.9*	5.76 ± 1.07

a) Mean ± SE (n=3).

b) *p<0.05, **p<0.01, ***p<0.001 vs Ovx-control group.

treatment. Slight hypercalcemia was noted in the high 1 α (OH)D₃ dose group. Serum Pi levels also increased significantly during repletion with active vitamin D₃ analogs, although there was no significant difference between intact and ovx-controls. The alkaline phosphatase activity was not affected by ovariectomy and/or treatment with any of the vitamin D₃ analogs.

Urinary Ca excretion decreased in ovx-controls compared with intact controls in 2, 4 and 6 months of observation, although these differences were not significant. In each vitamin D₃-treated group, remarkable increases were obtained for urinary Ca excretion. No differences were seen among the groups. A slight increase in urinary Pi was observed during treatment with vitamin D₃ analogs.

Table 3 shows the serum concentrations of iPTH and vitamin D metabolites. The PTH level after ovariectomy was two times higher than that in intact rats, and the administration of 1,24(R)(OH)₂D₃, 1,25(OH)₂D₃ and 1 α (OH)D₃ significantly prevented the increase at both the low and high doses. The 1,25(OH)₂D concentration showed significant lower values in the ovx-controls than that in the intact controls, whereas there were no significant changes in serum 25(OH)D and 24,25(OH)₂D concentrations between intact and ovx-controls. Serum 25(OH)D in all groups of vitamin D₃ treatment was signifi-

cantly lower than in intact rats. However, differences in effects of vitamin D₃ analogs were found in serum 24,25(OH)₂D and 1,25(OH)₂D levels. Serum 24,25(OH)₂D decreased significantly in the 1,24(R)(OH)₂D₃-treated groups alone as compared with the intact groups, while an increase tendency was observed in the 1,25(OH)₂D₃ and 1 α (OH)D₃-treated groups. There was also an increase in serum 1,25(OH)₂D₃ concentrations due to the administrations of 1,25(OH)₂D₃ and 1 α (OH)D₃, but in 1,24(R)(OH)₂D₃-treated groups, no difference was observed when compared with the ovx-control.

The chemical and morphological characteristics of femoral and tibial bones are given in Table 4. The ovx-rats had significantly heavier mean tibial dry weights than those of the intact group, and the further increases were found in vitamin D₃-treated groups. On the other hand, when compared with that of the intact rats, the ash content of the tibia was significantly lower in ovx-rats. Treatment with 1,24(R)(OH)₂D₃, 1,25(OH)₂D₃ and 1 α (OH)D₃ in ovx-rats was associated with dose-dependent significant increases in the ash content of the tibia. The femurs of the ovx-control group were slightly longer than those of the intact group, and the administration of vitamin D₃ analogs resulted in slight increases in length, but the difference was not significant. No differences were observed

Table 4. Effect of active vitamin D₃ analogs on femoral and tibial bones in ovariectomized rats

Group	Femur				Tibia		
	Dose ($\mu\text{g/kg}$)	length (mm)	mid-shaft cortical thickness (%)	mineral apposition rate ($\mu\text{m/day}$)	trabecular bone thickness (mm, $\times 45$)	dry weight (mg)	ash/dry weight (%)
Intact control	—	36.2 $\pm$ 0.2 ^a	34.1 $\pm$ 0.7 ^a	2.00 $\pm$ 0.31 ^b *	2.88 $\pm$ 0.05 ^b	512 $\pm$ 11 ^a **	66.9 $\pm$ 0.4 ^a ***
Ovx-control	—	36.9 $\pm$ 0.3	34.1 $\pm$ 1.0	1.07 $\pm$ 0.27	3.23 $\pm$ 0.07 ^{†††c}	587 $\pm$ 14	59.8 $\pm$ 0.6
1,24(R)(OH) ₂ D ₃	0.02	37.8 $\pm$ 0.3 ^b	35.8 $\pm$ 0.6	1.73 $\pm$ 0.09	2.67 $\pm$ 0.06	631 $\pm$ 20	62.9 $\pm$ 0.3***
	0.10	37.4 $\pm$ 0.3	36.7 $\pm$ 0.5*	2.20 $\pm$ 0.15*	3.08 $\pm$ 0.06 ^{††}	626 $\pm$ 22	64.5 $\pm$ 0.3***
1,25(OH) ₂ D ₃	0.02	37.2 $\pm$ 0.5	35.6 $\pm$ 0.8	2.10 $\pm$ 0.10*	3.33 $\pm$ 0.07 ^{†††}	615 $\pm$ 13	63.0 $\pm$ 0.6**
	0.10	37.1 $\pm$ 0.4	36.9 $\pm$ 0.9	2.45 $\pm$ 0.05*	3.61 $\pm$ 0.08 ^{†††}	657 $\pm$ 20*	65.1 $\pm$ 0.6***
1 α (OH)D ₃	0.02	37.0 $\pm$ 0.5	35.0 $\pm$ 1.2	1.70 $\pm$ 0.06	3.23 $\pm$ 0.08 ^{†††}	616 $\pm$ 20	62.7 $\pm$ 2.5*
	0.10	37.2 $\pm$ 0.3	37.4 $\pm$ 0.9*	1.73 $\pm$ 0.41	3.72 $\pm$ 0.07 ^{†††}	633 $\pm$ 14*	65.9 $\pm$ 0.5***

a) Mean $\pm$ SE (n=10). b)n=3.

b) *p<0.05, **p<0.01, ***p<0.001 vs Ovx-control group.

c) [†]p<0.05, ^{††}p<0.01, ^{†††}p<0.001 vs Intact control group. Fig. 1. Contact microradiographs of the proximal end of the tibia in ovariectomized rats treated with active vitamin D₃ analogs.

between the castrate and intact mid-shaft cortical thickness, and vitamin D₃ analogs showed a slight increase in this parameter of the femur. Mineral apposition rate was remarkably suppressed in the ovx-control group

compared with that in the intact group. In vitamin D₃-treated groups, an improvement in the mineral apposition rate was noted, i.e., the mineral apposition rate increased dose-dependently in the 1,24(R)(OH)₂D₃ group and in 1,25(OH)₂D₃ group. Also in the 1 α (OH)D₃ group, an slight improvement was observed.

Fig. 1 shows contact microradiographs (CMRs) of the proximal end of the tibia in each group. In the ovx-control group, remarkable reduction in trabecular bone of the metaphysis compared with the intact group was noted. In the medullary cavity of the diaphysis, no trabecular bone was found. In the 1 α (OH)D₃ 0.02 $\mu\text{g/kg}$ group, metaphyseal trabecular bone was only increased slightly, but the improvement was not remarkable. In the 1 α (OH)D₃ 0.10 $\mu\text{g/kg}$ group, the effect was remarkable and the bone specimen was very similar to that in the intact group. On the other hand, in the cases of 1,24(R)(OH)₂D₃ and 1,25(OH)₂D₃, treatment with both 0.02 $\mu\text{g/kg}$ and 0.10 $\mu\text{g/kg}$ induced the obvious increases in trabecular bone of the tibia.

The results in the trabecular bone thickness of tibia are shown in Table 4. In ovx-controls, the thickness significantly increased

when compared with intact controls. 1,24(R)(OH) $_2$ D $_3$ treatment apparently prevented the occurrence of thickened trabecular bone, while 1,25(OH) $_2$ D $_3$ and 1 α (OH)D $_3$ treatments caused a further increase in the thickness of the trabecular bone.

DISCUSSION

Although it has often been pointed out that loss of ovarian function, especially the estrogen deficiency, might be an important factor in the etiology of osteoporosis, there are few studies on castrated animals as experimental models of osteoporosis [1, 6, 10, 17]. There are no clear-cut qualitative and quantitative studies in young immature rats clarifying the role of estrogen in the bone metabolism. In addition, evaluation methods in the previous studies might not be sufficient to investigate the nature and pathogenesis of osteoporosis because they consisted mainly of analyses of bone components such as bone mass, ash and collagen contents, and only bone length and cortical thickness were measured for the morphological assessment.

The present study clearly shows that the long-term consequences of ovariectomy are development of mild osteoporosis in young immature rats. This was characterized by a reduction in ash content of the tibia and microradiographically by the disappearance of trabecular bone in the tibial metaphysis, although there were no changes in the femoral length and mid-shaft cortical thickness.

It has been thought that the effect of ovarian insufficiency on bone metabolism would involve an increased bone resorptive effect of parathyroid hormone and possible decreased synthesis of 1,25(OH) $_2$ D $_3$ in kidney [11]. In this point, Riggs *et al.* [16] demonstrated that the primary effect of sex hormones in osteoporosis was to decrease the increased level of bone resorption, perhaps by decreasing the responsiveness of bone to endogenous parathyroid hormone. Furthermore, DeLuca [4]

indicated that the serum level of 1,25(OH) $_2$ D $_3$ is low in patients with osteoporosis and also that both estrogen and progestogen are necessary to maintain a normal level of 1,25(OH) $_2$ D $_3$.

The present study strongly suggested that abnormal metabolism of vitamin D might exist in ovariectomized rats and it plays an important role in the development of osteoporosis. Although Lindgren *et al.* [10] have reported that there was no difference in serum Ca concentration between ovariectomized and intact rats, the serum Ca was significantly lower in ovx-controls than in intact controls in the present study. The reason why ovariectomy causes a reduction in serum Ca is not yet clear, but a decrease in intestinal Ca absorption appears to be one of the important factors. As shown in Table 3, there was a significant decrease in mean serum concentration of 1,25(OH) $_2$ D in ovariectomized rats, which suggested that Ca absorption from the intestine might be suppressed by ovariectomy. It is well established that intestinal Ca absorption is decreased in patients with osteoporosis [2]. However, serum 1,25(OH) $_2$ D level has been reported to be low by some investigators [4, 15], to be in normal range by others [12] in patients with osteoporosis and to be in the increased levels in ovariectomized rats [11], and opinions concerning this point are somewhat controversial. It is noteworthy that this study appeared to be the first report which clearly showed a decrease in serum 1,25(OH) $_2$ D concentration in castrated animals. The mechanism causing the decrease in serum 1,25(OH) $_2$ D due to ovariectomy is still unknown, but it may be the result of suppression of 25(OH)D $_3$ -1 α -hydroxylase activity in kidneys [3, 14], or stimulation of 1,25(OH) $_2$ D $_3$ metabolism in ovariectomized rats. Further studies are required to elucidate the causes of this phenomenon.

The above findings suggest that ovariectomy partially impairs intestinal absorp-

tion of Ca, increases bone resorption, and results in a negative Ca balance, which may contribute to the development of osteoporosis in rats.

The administration of 1,24(R)(OH)₂D₃, 1,25(OH)₂D₃ and 1 α (OH)D₃ dose-dependently increased serum and urinary Ca, decreased serum iPTH levels to the normal range and histologically prevented the development of osteoporosis. However, slight differences were noted among active vitamin D₃ analogs, i.e., metaphyseal trabeculae was increased in a dose-related manner in the case of 1,24(R)(OH)₂D₃ and 1,25(OH)₂D₃. On the other hand, 1 α (OH)D₃ was remarkably effective in the high dose group (0.10 μ g/kg), while little effect was noted in the low dose group (0.02 μ g/kg). These results seemed to be correlated to effect on serum Ca concentration. As shown in Table 1, high dose administration of all three vitamin D₃ analogs increased serum Ca levels near to that of intact rats. In 1 α (OH)D₃-treated group, there were some animals with slight hypercalcemia. In the low dose groups, improvement to the normal range of serum Ca was noted only in the case of 1,24(R)(OH)₂D₃ treatment. Ishizuka and coworkers [8] have reported that biological activities of 1,24(R)(OH)₂D₃ in intestinal Ca absorption and bone mobilization were almost equal or slightly weaker than those of 1,25(OH)₂D₃ in vitamin D-deficient rats. It is very interesting that the increase in serum Ca concentration due to 1,24(R)(OH)₂D₃ was more remarkable than that due to 1,25(OH)₂D₃ and 1 α (OH)D₃ in ovariectomized rats. At present, it is impossible to explain the reason for the apparent differences in the mode of action of 1,24(R)(OH)₂D₃ and 1,25(OH)₂D₃, and further studies are required to delineate more clearly the mechanism of action of 1,24(R)(OH)₂D₃.

When the three vitamin D₃ analogs are evaluated in terms of preventing a decrease in ash content of the tibia, 1,24(R)(OH)₂D₃ is as active as 1,25(OH)₂D₃ and 1 α (OH)D₃. How-

ever, it is extensively interesting that there are some morphological differences in the metaphyseal trabeculae of the tibia between the 1,24(R)(OH)₂D₃ group and 1,25(OH)₂D₃ or 1 α (OH)D₃ groups. Trabecular bone thickness was increased in the 1,25(OH)₂D₃ or 1 α (OH)D₃-treated groups, while the bone in the 1,24(R)(OH)₂D₃-treated group was very similar to that of the intact group. These results suggest that 1,24(R)(OH)₂D₃ may play an important role in the bone different from that of 1,25(OH)₂D₃ or 1 α (OH)D₃.

The present study shows some evidences suggesting that active vitamin D₃ analogs are remarkably effective, and above all 1,24(R)(OH)₂D₃ is more effective than 1,25(OH)₂D₃ and 1 α (OH)D₃ in the treatment of osteoporosis.

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REFERENCES

1. Aitken, J. M. and Anderson, J. B. 1972. Osteoporosis after oophorectomy in the mature female rat and the effect of oestrogen and/or progestogen replacement therapy in its prevention. *J. Endocrinol.* 55: 79-87.
2. Bullamore, J. R., Gallagher, J. C., Wilkinson, R., Nordin, B. E. C., and Marshall, D. H. 1970. Effect of age on calcium absorption. *Lancet* 1: 535-537.
3. Castillo, L., Tanaka, Y., DeLuca, H. F., and Sunde, M. L. 1977. The stimulation of 25-hydroxyvitamin D₃-1 α -hydroxylase by estrogen. *Arch. Biochem. Biophys.* 179: 211-217.
4. DeLuca, H. F. 1977. Vitamin D metabolism. *Clin. Endocrinol.* (suppl.) 7: 1-17.
5. Eisman, J. A., Hamsra, A. J., Kream, B. E. and DeLuca, H. F. 1976. A sensitive precise and convenient method for determination of 1,25-dihydroxyvitamin D in human plasma. *Arch. Biochem. Biophys.* 176: 235-243.
6. Furguson, H. W., and Hartles, R. L. 1970. The combined effects of calcium deficiency and ovariectomy on the bones of young adult cats. *Calcif. Tissue Res.* (suppl.): 140-141.
7. Haas, H. G., Dambacher, M. A., Guncaga, J.,

- Lauffenburger, T., Lammle, B., and Olah, J. 1979. 1,25(OH)₂-vitamin D₃ in osteoporosis- a pilot study. *Horm. Metab. Res.* 11: 168-171.
8. Ishizuka, S., Ohnuma, N., Kiyoki, M., Yamaguchi, H., and Hashimoto, Y. 1981. Biological activities, metabolism of action of 1 α ,24-dihydroxyvitamin D₃. *Bone Metabolism* 14: 159-169 (in Japanese).
9. Izawa, Y., Makita, T., Hino, S., Hashimoto, Y., Kushida, K., Inoue, T., and Orimo, H. 1981. Immobilization osteoporosis and active vitamin D: Effect of active vitamin D analogs on the development of immobilization osteoporosis in rats. *Calcif. Tissue Int.* 33: 623-630.
10. Lindgren, J. U., and Lindholm, T. S. 1979. Effect of 1-alpha-hydroxyvitamin D₃ on osteoporosis in rats induced oophorectomy. *Calcif. Tissue Int.* 27: 161-164.
11. Lindgren, U., and DeLuca, H. F. 1982. Role of parathyroid hormone and 1,25-dihydroxyvitamin D₃ in the development of osteopenia in oophorectomized rats. *Calcif. Tissue Int.* 34: 510-514.
12. Nordin, B. E. C., Peacock, M., Crilly, R. G., and Marshall, D. H. 1979. Calcium absorption and plasma 1,25(OH)₂D levels in post-menopausal osteoporosis. pp. 99-106. In: Vitamin D, Basic Research and its Clinical Application. (Norman, A. W. et al. eds.), Walter de Gruyter, Berlin, New York.
13. Orimo, H., Fujita, T., and Yoshikawa, M. 1971. Effect of calcitonin on the development of immobilization osteoporosis in rats. *Endocrinol. Jpn.* 18: 117-121.
14. Orimo, H., Fujita, T., and Yoshikawa, M. 1972. Increased sensitivity of bone to parathyroid hormone in ovariectomized rats. *Endocrinology* 90: 760-763.
15. Riggs, B. L., Gallagher, J. C., and DeLuca, H. F. 1979. Osteoporosis and age-related osteopenia: Evaluation of possible role of vitamin D endocrine system in pathogenesis of impaired intestinal calcium absorption. pp. 107-113. In: Vitamin D, Basic Research and its Clinical Application. (Norman, A. W. et al. eds.), Walter de Gruyter, Berlin, New York.
16. Riggs, B. L., Jowsey, J., Goldsmith, R. S., Kelly, P. J., Hoffman, D. L., and Anderson, C. D. 1972. Short and long-term effects of estrogen and synthetic anabolic hormone on post-menopausal osteoporosis. *J. Clin. Invest.* 51: 1659-1663.
17. Saville, P. D. 1969. Changes in skeletal mass and fragility with castration in the rats; A model of osteoporosis. *J. Am. Geriatr. Soc.* 17: 155-166.
18. Taylor, C. M., Hughes, S. E., and Desilva, P. 1976. Competitive protein binding assay for 24,25-dihydroxycholecalciferol. *Biochem. Biophys. Res. Commun.* 70: 1243-1249.

要 約

卵巣摘出ラットの骨粗鬆化に対する活性型ビタミン D₃ の効果: 伊沢義弘・蒔田徳太郎・一木彦三¹⁾ (帝人 株式会社 生物医学研究所, ¹⁾ 日本獣医畜産大学獣医第二外科学教室) —— 6 週齢ラットの卵巣を摘出し, 6 カ月間正常飼料で飼育したところ, 脛骨灰分量が減少し, 骨幹端における海綿骨梁が消失したが, これは血中 1, 25(OH)₂D₃ 濃度の低下にもとづく腸からの Ca 吸収の低下あるいは血中上皮小体ホルモン (PTH) 分泌亢進による骨吸収の増加, または両者の複合によると考えられた。活性型の 1, 24(R) (OH)₂D₃ あるいは 1, 25(OH)₂D₃, 1 α (OH) D₃ の投与により, PTH 分泌は抑制され, 投与量に相関して血清 Ca 値が正常化し, 骨粗鬆化が抑制された。これらの成績から, 卵巣機能の低下が骨粗鬆化発生の要因であり, 活性型ビタミン D₃ が骨粗鬆化の防止に有効であることが示唆された。