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2β-(3-HYDROXYPROPOXY)-1α,25-DIHYDROXYVITAMIN D_3 (ED-71), PREVENTIVE AND THERAPEUTIC EFFECTS ON BONE MINERAL LOSS IN OVARIECTOMIZED RATS

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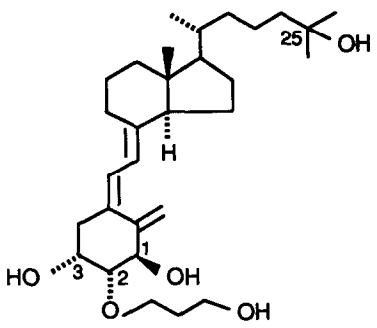
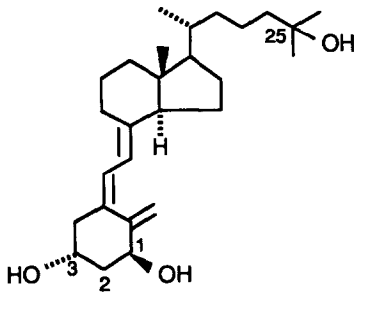
Abstract: 2β-(3-Hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 (ED-71) improved bone mineral density and mechanical bone strength in the pre-osteoporosis and osteoporosis model rats made by ovariectomy more effectively than 1α,25-dihydroxyvitamin D_3.

Attention has been focused recently on the structural modification of 1α,25-dihydroxyvitamin D_3[1,25$(OH)_2D_3$], a hormonally active form of vitamin D_3, in order to find more active compounds than 1,25$(OH)_2D_3$ or to separate its differentiation inducing activity from the calcemic activity[1-6]. In a previous paper[7], we reported regulatory calcemic metabolism activities of 2β-(3-hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 [ED-71] (Chart 1), an analogue of 1,25$(OH)_2D_3$ with modified A ring structure.



2β-(3-Hydroxypropoxy)-1α,25-dihydroxyvitamin D3
(ED-71)



1α,25-Dihydroxyvitamin D3
(1,25(OH)2D3)

Chart 1

As summarized in Table 1, this compound behaves similarly to $1,25(OH)_2D_3$ in the *in vivo* measured intestinal calcium absorption and bone calcium resorption. Its half-life in plasma is longer than that of $1,25(OH)_2D_3$ because of its stronger binding to vitamin D binding protein (DBP).

Table 1. Biological Properties of ED-71 and 1,25(OH)$_2$D$_3$

Biological property	ED-71	1,25(OH)$_2$D$_3$
In vitro		
Binding potency to DBP	2.7	1
Binding potency to VDR	1/8	1
Bone resorption	Weak stimulation	Stimulation
In vivo		
Intestinal Ca absorption	Stimulation	Stimulation
Bone Ca mobilization	Stimulation	Stimulation
Half-life in plasma	Long	Short

These results suggest that ED-71 is a promissing candidate for therapy of osteoporosis. In this paper, we describe the preventive and therapeutic effects of ED-71 on bone mineral loss in pre-osteoporosis and osteoporosis ovariectomized rat models (OVX).

Experimental : Figure 1 shows the protocol for the experiments 1 and 2 used in the study of preventive and therapeutic effects of ED-71 using the OVX rats, respectively. $1,25(OH)_2D_3$ was used as a standard compound.

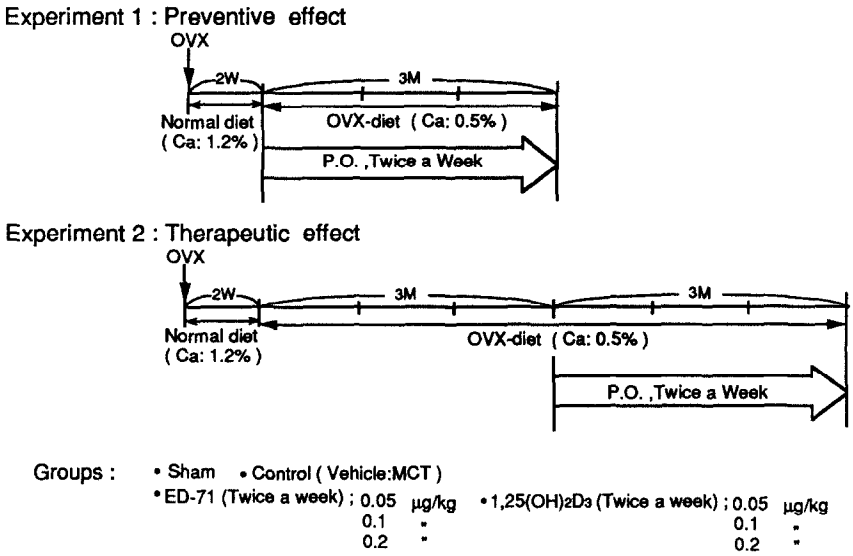


Fig.1 Protocol for Experiments 1(Preventive Effect) and 2(Therapeutic Effect)

Fischer strain female 9 months old rats were ovariectomized and fed *ad libitum* normal diet (calcium content: 1.2%) for 2 weeks. These rats are denoted as pre-osteoporosis model. In the experiment 1, the rats were fed OVX-diet (calcium content: 0.5%) with peroral administration of 0.05, 0.1 or 0.2 μg/kg of ED-71 or $1,25(OH)_2D_3$ twice a week for 3 months. On the other hand, in the experiment 2, rats were fed the OVX-diet for 3 months before administration of the compounds. These rats are denoted as osteoporosis model rats. The Sham operated group was fed the OVX-diet without administration of the compounds and the control group was fed the OVX-diet with oral administration of medium chain triglyceride (MCT) as vehicle.

The rats were sacrificed after each final stage. Bone mineral density (BMD) of spine (L2-L5) and tibia was measured by a dual energy X-ray absorptiometer (DEXA, Aloka DCS-600, Tokyo, Japan) and mechanical bone strength (MBS) of femur was measured by a computerized bone measuring apparatus MZ-501D (Maruto Testing Machine Co., Tokyo, Japan). Concentrations of calcium, phosphorus and parathyroid hormone (PTH) and alkaline phosphatase activity in plasma were measured by the respective conventional methods and all the values were within the respective normal ranges in the both experiments.

BMD and MBS in the Experiment 1 (Preventive Effect): As shown in Fig. 2, ED-71 increased BMD of spine and MBS in the experiment 1 in a dose-dependent fashion without inducing hypercalcemia. On the other hand, significant increase of BMD and MBS

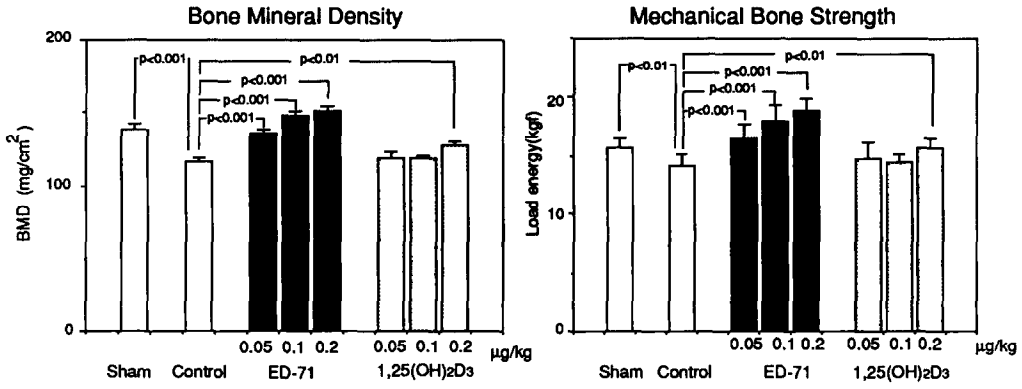


Fig. 2 Spinal Bone Mineral Density and Femoral Mechanical Bone Strength in the Pre-Osteoporosis Model Rats Given Orally Either ED-71 or 1,25(OH)2D3 for Three Months (Experiment 1)

in the $1,25(OH)_2D_3$ groups was observed only at a dose of 0.2 μg/kg but not in the other two groups. Similar results in BMD of tibia were also observed in all the groups, through the data are not shown. The results suggest that ED-71 has more positive effects than $1,25(OH)_2D_3$ on BMD and MBS in the pre-osteoporosis rat model.

BMD and MBS in the Experiment 2 (Therapeutic Effect): As shown in Fig. 3, significant increase of BMD and MBS was observed in the group given 0.2 μg/kg of ED-71 of the experiment 2. On the other hand, no significant increase was observed in all the three groups

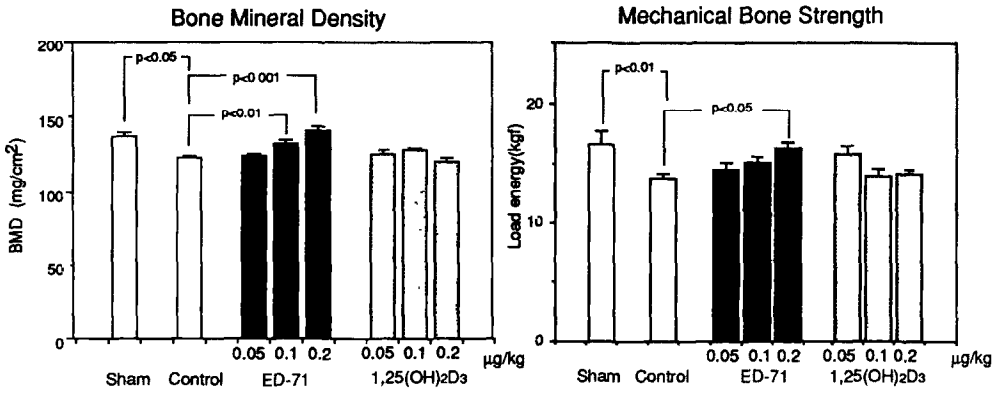


Fig. 3 Spinal Bone Mineral Density and Femoral Mechanical Bone Strength in the Osteoporosis Model Rats Given Orally Either ED-71 or 1,25(OH)2D3 for Three Months (Experiment 2)

given $1,25(OH)_2D_3$. The results also suggest that ED-71 has more positive effect than $1,25(OH)_2D_3$ on BMD and MBS in the osteoporosis rat model.

Conclusion: All the results in this report show that ED-71 improved BMD and MBS in the pre-osteoporosis and osteoporosis rat models more effectively than $1,25(OH)_2D_3$. Since the results of the experiment 1 show more efficacy than those of the experiment 2, the preventive effect of ED-71 and $1,25(OH)_2D_3$ on bone mineral loss seems to be better than the respective therapeutic effect. We conclude from the present studies that ED-71 is a promissing candidate for a prevention of osteoporosis.

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