· ED-71, A NOVEL VITAMIN D ANALOG, PROMOTES BONE FORMATION AND INHIBITS BONE RESORPTION AFTER BONE MARROW ABLATION

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INTRODUCTION:
1,25-Dihydroxyvitamin D3 (Vitamin D) plays an essential role in bone homeostasis. A novel vitamin D analog 2-ß-(3-hydroxypropoxy)-1a,25-dihydroxyvitamin D3 (ED-71) have high affinity with vitamin D binding protein and have greater activity than vitamin D in preventing bone loss in ovariectomized rat. However, the effect of ED-71 on bone remodeling including bone formation and resorption remains to be elucidated. Bone marrow ablation in the long bone causes vigorous new bone formation within the first week and then subsequent rapid bone resorption in the second week to regenerate bone marrow with normal level of trabecular bones. It is a highly reproducible in vivo assay to evaluate bone remodeling. The purpose of this study was to evaluate the effect of ED-71 on bone remodeling in vivo by using mice bone ablation model.

METHODS:
ICR female mice between 6 and 7 weeks of age were used for these experiments in accordance with the guidelines for animal studies in our institute. They were divided into ED-71 treatment group and control group. Longitudinal incisions were made on the bilateral knees of each mouse to expose the femoral condyle through parapatellar approach. A hole was made at the intercondylar notch of the femur with dental drill and then the hole was enlarged and the content of the bone marrow removed gradually by using dental drills. Finally a 0.6mm diameter Kirschner wire was inserted up to the proximal end of the femur to ensure completion of marrow ablation by radiography. After all, Kirschner wire was removed and the skin was closed. Mice were divided into four groups for two experiments.

[experiment 1: ED-71 administration the day after operation]
The day after operation, mice of control group and ED-71 treatment group were intraperitoneally administered once vehicle or ED-71 (0.8µg/kg body weight) respectively. Mice were sacrificed 3,5,7,14 days after surgery.

[experiment 2: ED-71 administration 8 days after operation]
Mice of control group and ED-71 treatment group were intraperitoneally administered once vehicle or ED-71 (0.8µg/kg body weight) 8 days after operation and mice were sacrificed 14 days after operation. Right side femurs were fixed with 4% paraformaldehyde, decalcified with 20% ethylenediamine tetraacetic acid and embedded in paraffin. Left side femurs were fixed with 70% ethanol and observed by micro CT analysis using µCT 40(SCANCO MEDICAL). All samples were scanned the ablated cylindrical area, which had a diameter of 600µm and a height of 800µm, 400µm proximal to the growth plate.

RESULTS:
[experiment 1]

First, ED-71 was injected once the day after bone marrow ablation to elucidate the effect of ED-71 on rapid bone formation during bone remodeling. Histological examination showed that ablated bone marrow in ED-71 treatment group were filled with more abundant matrix compared with control 3 and 5 days after surgery. As trabecular bone volume reaches at its maximal level 7 days after surgery, more abundant and thicker newly formed trabecular bones were observed in ED-71 treatment group. Micro CT analysis of the mice sacrificed 7 days after surgery revealed that bone volume/tissue volume (BV/TV) was 50% more in ED-71 treatment group. Trabecular bone thickness (Th.Th) was also increased in ED-71 treatment group. Histological examination revealed osteoclast number/bone surface (N.Oc/BS) was not affected by ED-71 treatment. By 14 days after surgery, when newly formed trabecular bone was resorbed and remodeled, BV/TV was similar between ED-71 treatment group and control group.

[experiment 2]
Second, ED-71 was injected once 8 days after operation to elucidate the effect of ED-71 on bone resorption. Micro CT analysis revealed that BV/TV was 70% more in ED-71 treatment group compared with control group 14 days after operation. Body weight was similar among all four groups in both experiment 1 and 2.

DISCUSSION:
Present study reported that administration of ED-71 immediately after bone marrow ablation raised the maximal level of bone volume. Histological results, that N.Oc/BS was not affected by ED-71 administration, indicated that at least ED-71 did not inhibit bone formation as a result of suppression of bone formation/resorption coupling. The data that BV/TV was not affected by ED-71 injected immediately after the operation indicated that trabecular bone remodeling was normally carried out after the effect of ED-71 was abrogated. Moreover, ED-71 injection 8 days after bone marrow ablation increased BV/TV 14 days after the operation, passibly because of reduced bone resorption activity in the phase of bone remodeling. Whereas most anti-osteoporosis drugs target bone resorption, there are few reports about low molecular weight compounds that enhance bone formation. ED-71 may be expected as a novel factor that has both promoting effect of bone formation and inhibiting effect of bone resorption. ED-71 could be useful for wide applications not only for the treatment of osteoporosis, but also for the purpose of enhancing bone formation such as fractured bone repair or reconstructing bone defect.

We concluded that ED-71 increased bone volume in both bone formation phase and bone resorption phase in the region of rapid bone turn over after bone marrow ablation.