# Effect of 24,25-Dihydroxyvitamin $D_3$ in Osteoclasts (43410)

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*Abstract.* **Previous results demonstrated that the administration of pharmacological doses of 24,25-dihydroxyvitamin $D_3$ ($24,25(OH)_2D_3$) to animals reduces bone resorption and increases bone volume with a decrease in osteoclast number. In order to clarify whether $24,25(OH)_2D_3$ has an effect to inhibit osteoclastic bone resorption, the effect of $24,25(OH)_2D_3$ on the formation and function of osteoclastic cells was examined *in vitro*. Treatment of hemopoietic blast cells, which are progenitors of osteoclasts, with parathyroid hormone (PTH) or 1,25-dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$) stimulated the formation of osteoclast-like multinucleated cells in a dose-dependent manner. Although $24,25(OH)_2D_3$ in itself had little effect on osteoclast-like multinucleated cells formation, it inhibited the stimulatory effect of PTH on the formation of osteoclastic cells. In addition, $24,25(OH)_2D_3$ also inhibited the stimulation of resorption pit formation by osteoclasts under stimulation with PTH. In contrast, $1,25(OH)_2D_3$ stimulated the formation and function of osteoclastic cells even at low concentrations, and the effect was additive to PTH. These results could not be explained by either an agonistic or antagonistic effect of $24,25(OH)_2D_3$ on $1,25(OH)_2D_3$, and are consistent with the assumption that $24,25(OH)_2D_3$ has a unique inhibitory effect on the formation and function of osteoclasts. Because $24,25(OH)_2D_3$ is shown to stimulate the degradation of $1,25(OH)_2D_3$ and because the formation of $24,25(OH)_2D_3$ is stimulated by $1,25(OH)_2D_3$ not only in the kidney but also in many of its target tissues, including bone, the inhibitory effect of $24,25(OH)_2D_3$ on osteoclastic bone resorption may play a role in the local modulation of the actions of osteotropic hormones in bone.** [P.S.E.B.M. 1992, Vol 200]

Although 24,25-dihydroxyvitamin $D_3$ ($24,25(OH)_2D_3$) is the most abundant dihydroxylated metabolite of vitamin $D_3$ when the vitamin D supply is sufficient, the biological role of $24,25(OH)_2D_3$ in the regulation of calcium (Ca) metabolism has been questioned. However, when pharmacological amounts of $24,25(OH)_2D_3$ were administered to 1$\alpha$-hydroxyvitamin $D_3$-induced hypercalcemic rats, there was a reduction in serum Ca concentration (1). Subsequent studies by us revealed that $24,25(OH)_2D_3$ reduces serum 1,25-dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$) levels by enhancing the metabolic degradation of $1,25(OH)_2D_3$ without affecting the production of $1,25(OH)_2D_3$ (2, 3). Those studies demonstrated that $24,25(OH)_2D_3$ can affect Ca metabolism at least in part through its effect on $1,25(OH)_2D_3$ metabolism. However, controversy remained as to whether $24,25(OH)_2D_3$ has its own effect on bone and Ca metabolism.

Recently, it was reported that the administration of pharmacological doses of $24,25(OH)_2D_3$ to animals reduces bone resorption and increases bone volume with a decrease in osteoclast number (4–6). Those results suggested that $24,25(OH)_2D_3$ may inhibit osteoclastic bone resorption by affecting the formation and/or function of osteoclasts. However, the results obtained by those *in vivo* experiments could also be explained by its effect on $1,25(OH)_2D_3$ metabolism. Thus, the present study was undertaken to clarify whether $24,25(OH)_2D_3$ has its own effect on the formation or function of osteoclasts. In order to eliminate the possibility that the effect is due to a stimulation of $1,25(OH)_2D_3$ metabolism or to an interference with $1,25(OH)_2D_3$ action, the direct *in vitro* effect of $24,25(OH)_2D_3$ on osteoclast formation and function was examined under stimulation with parathyroid hormone (PTH).

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## Materials and Methods

**Assay of Osteoclast-Like Multinucleated Cell Formation.** Six-week-old female BDF1 mice were given 150 mg/kg of 5-florouracil intravenously. Spleen cells were harvested at 4 days after the injection and cell suspensions were prepared. Aliquots of $1.8 \times 10^6$/ml spleen cells were plated in culture dishes in 1 ml of $\alpha$-minimum essential medium containing 1.2% methylcellulose, 50 units/ml of interleukin 3, 30 ng/ml of interleukin 6, 10 mg of deionized bovine serum albumin and 30% fetal bovine serum. After about 5 days, hemopoietic blast cell colonies were lifted from the dish and plated into each well of a 48-well microplate at a density of $8 \times 10^3$/ml in 200 $\mu$l of $\alpha$-minimum essential medium supplemented with 5% fetal bovine serum and 50 units/ml of interleukin 3. Four days later, cells were treated with either $10^{-8}$ *M* $1,25(OH)_2D_3$ or PTH(1-34) in the presence or absence of $10^{-6}$ *M* $24,25(OH)_2D_3$, and cultured for 4 more days. The cells adherent to the plates at the end of experiments were stained for tartrate-resistant acid phosphatase. The cells containing three or more nuclei were counted as multinucleated cells (MNC) (7,8).

**Assay of Resorption Pit Formation.** Bone cells containing osteoclasts were desegregated from tibia and femur of 9-day-old ICR mice. These cells at a density of $5 \times 10^6$/ml were spotted on dentine slices in 100 $\mu$l of $\alpha$-minimum essential medium supplemented with 5% fetal bovine serum. After incubation for 2 hr, cells on dentine slices were treated with $10^{-8}$ *M* $1,25(OH)_2D_3$ or PTH with or without $10^{-6}$ *M* $24,25(OH)_2D_3$. Five days later, the cells were removed and the dentine slices were stained with acid hematoxylin. The area of the resorption pits was determined by a net micrometer disk (1 mm × 1 mm) in the eye piece of a light microscope (9).

## Results

Effects of varying concentrations of PTH, $1,25(OH)_2D_3$, and $24,25(OH)_2D_3$ on the formation of tartrate-resistant acid phosphatase-positive MNC is shown in Figure 1. Treatment of hemopoietic blast cell cultures with PTH(1-34) as well as $1,25(OH)_2D_3$ stimulated osteoclast-like MNC formation in a dose-dependent manner, and the maximal effect was observed at $10^{-8}$ *M* of PTH(1-34) and $1,25(OH)_2D_3$. In contrast, up to $10^{-7}$ *M* $24,25(OH)_2D_3$ had no significant effect, and $10^{-6}$ *M* $24,25(OH)_2D_3$ showed only marginal stimulation of MNC formation. Because $24,25(OH)_2D_3$ can also bind to $1,25(OH)_2D_3$ receptors with much lower affinity than that of $1,25(OH)_2D_3$, the slight stimulatory effect of the high concentration of $24,25(OH)_2D_3$ on MNC formation appears to be due to the interaction of $24,25(OH)_2D_3$ with $1,25(OH)_2D_3$ receptors. As shown in Figure 2, when $24,25(OH)_2D_3$ was added together with PTH(1-34), the stimulatory effect of PTH(1-34)

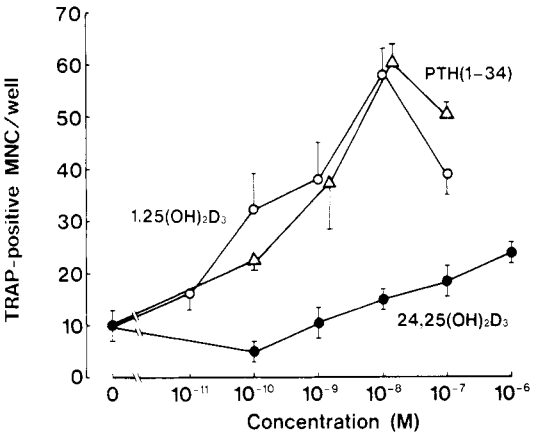


**Figure 1.** Dose response of the effect of PTH(1-34), $1,25(OH)_2D_3$, and $24,25(OH)_2D_3$ on the formation of osteoclast-like tartrate-resistant acid phosphatase (TRAP)-positive MNC. Hemopoietic blast cell colonies were obtained as described in Materials and Methods. On the fourth day of culture, cells were treated with the indicated concentrations of PTH(1-34), $1,25(OH)_2D_3$, or $24,25(OH)_2D_3$. Four days later, cells were stained for TRAP, and cells containing three or more nuclei were counted as MNC. Data are expressed as means ± SE for four cultures. $1,25(OH)_2D_3$ ($10^{-10}$ *M*) and PTH(1-34) ($10^{-9}$ *M*) significantly increased the number of MNC, and $10^{-8}$ *M* $1,25(OH)_2D_3$ and PTH(1-34) exhibited maximal effects. In contrast, up to $10^{-7}$ *M* $24,25(OH)_2D_3$ showed no significant effect, and $10^{-6}$ *M* $24,25(OH)_2D_3$ caused only a small increase in TRAP-positive MNC.

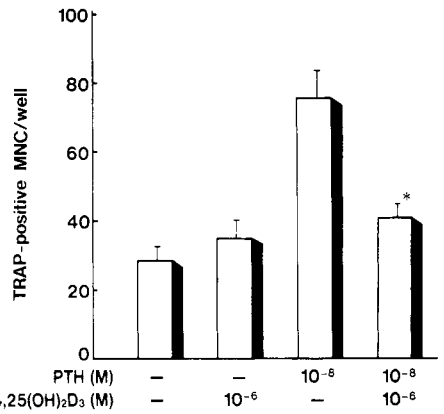


**Figure 2.** Effect of $24,25(OH)_2D_3$ on the formation of TRAP-positive MNC stimulated by PTH(1-34). The formation of osteoclast-like MNC was assayed as described in the legend to Figure 1. $24,25(OH)_2D_3$ ($10^{-6}$ *M*) significantly reduced the formation of TRAP-positive MNC formation stimulated by $10^{-8}$ *M* PTH(1-34). Data are means ± SE for four cultures. Asterisk indicates a significant difference from cultures with PTH(1-34) alone ($P < 0.01$).

on tartrate-resistant acid phosphate-positive MNC formation was significantly inhibited. Thus, although $24,25(OH)_2D_3$ in itself has very little effect on osteoclast-like cell formation, it inhibits the stimulatory effect of PTH on the formation of osteoclastic cells.

In order to clarify whether $24,25(OH)_2D_3$ also affects the resorptive function of osteoclasts, the effect of $24,25(OH)_2D_3$ on resorption pit formation on dentine slices was examined under stimulation with PTH(1-34). As shown in Figure 3, the addition of $10^{-8}$ *M* PTH(1-34) to the culture markedly stimulated the formation

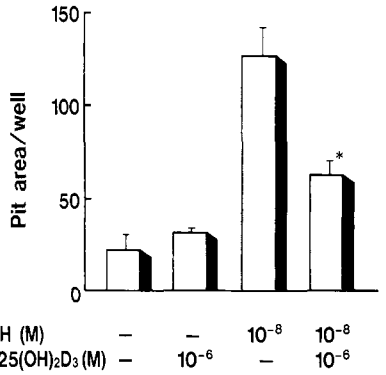


**Figure 3.** Effect of 24,25(OH)$_2$D$_3$ on the formation of resorption pits on dentine slices stimulated by PTH(1-34). Bone cells containing osteoclasts were desegregated from tibia and femur of 9-day-old mice. These cells were spotted on dentine slices and incubated for 2 hr as mentioned in Materials and Methods. They were then treated with $10^{-8}$ *M* PTH(1-34) with or without $10^{-6}$ *M* 24,25(OH)$_2$D$_3$. Five days later, the area of the resorption pits was determined. 24,25(OH)$_2$D$_3$ significantly reduced the formation of resorption pits stimulated by PTH(1-34). Data are means ± SE for four cultures. Asterisk indicates a significant difference from cultures with PTH(1-34) alone ($P < 0.01$).

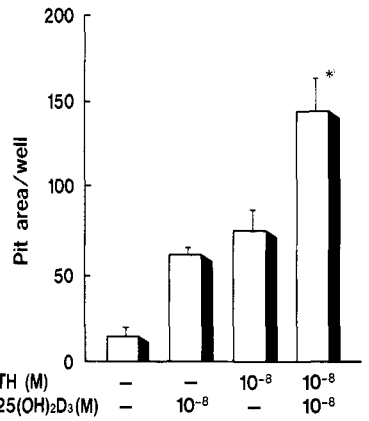


**Figure 4.** Interaction of 1,25(OH)$_2$D$_3$ and PTH(1-34) on the formation of resorption pits on dentine slices. The areas of resorption pits formed on dentine slices were determined as described in the legend to Figure 3. 1,25(OH)$_2$D$_3$ ($10^{-8}$ *M*) and PTH(1-34) exhibited an additive effect on resorption pit formation. Data are means ± SE for four cultures. Asterisk indicates a significant difference from cultures with 1,25(OH)$_2$D$_3$ or PTH(1-34) alone ($P < 0.01$).

of resorption pits. Furthermore, when $10^{-6}$ *M* 24,25 (OH)$_2$D$_3$ was added along with PTH(1-34), the pit formation by mouse osteoclasts was again inhibited. In contrast, 1,25(OH)$_2$D$_3$ stimulated pit formation, and the treatment of cultures with $10^{-8}$ *M* 1,25(OH)$_2$D$_3$ together with PTH(1-34) exhibited an additive effect on the formation of resorption pits (Fig. 4).

## Discussion

The present studies demonstrate that 24,25(OH)$_2$D$_3$ inhibits the formation and function of osteoclastic cells stimulated by PTH(1-34). These results are in good agreement with the previous *in vivo* observations that the administration of large amounts

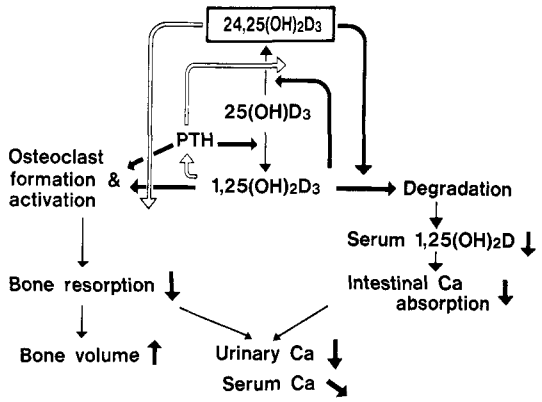


**Figure 5.** Schematic illustration of synthesis, metabolism, and actions of PTH, 1,25(OH)$_2$D$_3$, and 24,25(OH)$_2$D$_3$ on bone and Ca metabolism. 24,25(OH)$_2$D$_3$ stimulates the degradation of 1,25(OH)$_2$D$_3$ and inhibits the effect of PTH and 1,25(OH)$_2$D$_3$ on osteoclast formation and function. These effects of 24,25(OH)$_2$D$_3$ may lead to reduced bone resorption with an increase in bone volume as well as a reduction in urinary and serum Ca level. Thus, the effect of 1,25(OH)$_2$D$_3$ can be modulated by 24,25(OH)$_2$D$_3$ at the target tissues including bone, where the synthesis of 24,25(OH)$_2$D$_3$ is stimulated by 1,25(OH)$_2$D$_3$. In contrast, elevation of PTH stimulates the formation of 1,25(OH)$_2$D$_3$, and PTH in concert with 1,25(OH)$_2$D$_3$ stimulates the formation and function of osteoclasts to enhance bone resorption. The effect of PTH to mobilize Ca from bone can be fully exerted by its effect to suppress the renal production of 24,25(OH)$_2$D$_3$ and to reduce its serum level.

of 24,25(OH)$_2$D$_3$ reduces resorption surface and osteoclast number in rats, rabbits, and beagle dogs (4–6). Although 24,25(OH)$_2$D$_3$ in itself showed a slight stimulatory effect on the formation and function of osteoclastic cells, the stimulatory effect of 24,25(OH)$_2$D$_3$ at a high concentration appears to be due to its interaction with 1,25(OH)$_2$D$_3$ receptors. The same concentration of 24,25(OH)$_2$D$_3$ inhibited osteoclast formation and function stimulated not only by 1,25(OH)$_2$D$_3$ (data not shown) (10), but also by PTH(1-34). In addition, 1,25(OH)$_2$D$_3$ stimulated the formation and function of osteoclastic cells even at low concentrations, and the effect was additive to that of PTH(1-34). Thus, the inhibitory effects of 24,25(OH)$_2$D$_3$ on osteoclastic cells could not be explained by either its weak agonistic effect for 1,25(OH)$_2$D$_3$ or its antagonistic effect against 1,25(OH)$_2$D$_3$. These results are consistent with the assumption that 24,25(OH)$_2$D$_3$ has a unique inhibitory effect on the formation and function of osteoclasts.

We have previously demonstrated that 24,25 (OH)$_2$D$_3$ stimulates the degradation of 1,25(OH)$_2$D$_3$ and reduces its serum level (2, 3). Although both PTH and 1,25(OH)$_2$D$_3$ stimulates osteoclastic bone resorption, 1,25(OH)$_2$D$_3$ inhibits the synthesis of PTH and stimulates the renal production of 24,25(OH)$_2$D$_3$ (Fig. 5). In view of the fact that 1,25(OH)$_2$D$_3$ stimulates the synthesis of 24,25(OH)$_2$D$_3$ not only in the kidney but also in many of its target organs, including bone (11), the local concentration of 24,25(OH)$_2$D$_3$ could become much higher than that in the systemic circulation. Thus,

the inhibitory effect of $24,25(OH)_2D_3$ on the stimulation of osteoclastic bone resorption as well as the stimulation of the degradation of $1,25(OH)_2D_3$ may play an important role in the local regulation of $1,25(OH)_2D_3$ action in its target tissues. In addition, the fact that PTH stimulates the synthesis of $1,25(OH)_2D_3$ and inhibits the production of $24,25(OH)_2D_3$ in the kidney suggests that, in Ca-deficient circumstances, the stimulatory effect of PTH on Ca mobilization from bone can be fully exerted by suppressing the production of $24,25(OH)_2D_3$ (Fig. 5). Thus, the present results and the previous observations suggest that there is a mutual regulation of the synthesis, metabolism, and actions of Ca-regulating hormones for the maintenance of bone and Ca metabolism, and that the inhibitory effect of $24,25(OH)_2D_3$ on osteoclastic bone resorption may play a role in the local modulation of the actions of osteotropic hormones in bone.


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