BONE RESORBING ACTIVITIES OF 24-HYDROXY STEREOISOMERS OF 24-HYDROXYVITAMIN D_3 AND 24,25-DIHYDROXYVITAMIN D_3†

by

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Summary

R and S isomers of 24-OH-D_3 and 24,25-$(OH)_2D_3$ were tested for their effects on bone resorption in vitro. 24(R),25-$(OH)_2D_3$ was more active than 24(S),25-$(OH)_2D_3$. Likewise, 24(R)-OH-D_3 was more active than 24(S)-OH-D_3. The bone resorbing activity of 24(R)-OH-D_3 was equivalent to that of 25-OH-D_3; 24(R),25-$(OH)_2D_3$ was somewhat less potent. The results indicate that there is discrimination between the isomers of these compounds at the level of the responding tissue.

The preparation of the R and S isomers of synthetic 24-hydroxyvitamin D_3 (24-OH-D_3)[1] and 24,25 dihydroxyvitamin D_3 (24,25-$(OH)_2D_3$) has been reported recently (1,2). These compounds are of interest because 24,25-$(OH)_2D_3$ is the major product of 25-hydroxyvitamin D_3 (25-OH-D_3) in normal (non vitamin D-deficient) animals (3). The analogs having the 24 hydroxyl group, but lacking the 25 hydroxyl could be useful in defining the function of the 24-hydroxyl group. The R and S derivatives of both 24-OH-D_3 and 24,25-$(OH)_2D_3$ were not remarkably different in their effects on intestinal calcium trans-

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[1]Abbreviations: 25-hydroxyvitamin D_3, 25-OH-D_3; 24,25-dihydroxyvitamin D_3, 24,25-$(OH)_2D_3$; 24-hydroxyvitamin D_3, 24-OH-D_3.

port in intact, D-deficient rats (1,2,4). However, 24(R),25-$(OH)_2D_3$ and 24(R)-OH-D_3 were markedly superior to the S enantiomers in stimulating bone calcium mobilization, in antiarchitic activity and in elevating serum phosphorus. Nephrectomy effectively eliminated activity, suggesting a requirement for 1-hydroxylation. Factors which could theoretically lead to discrimination between the isomers would include distribution factors such as transport to and rate of removal from specific tissues. Alternatively, the specificity could lie at the level of a tissue receptor. The relative contributions of these two mechanisms can best be evaluated in vitro. In previous studies, organ cultures of fetal rat bone have been shown to be sensitive to the bone resorbing effects of vitamin D metabolites (5-10). We have therefore examined the effects of the R and S isomers of 24-OH-D_3 and 24,25-$(OH)_2D_3$ in this in vitro system.

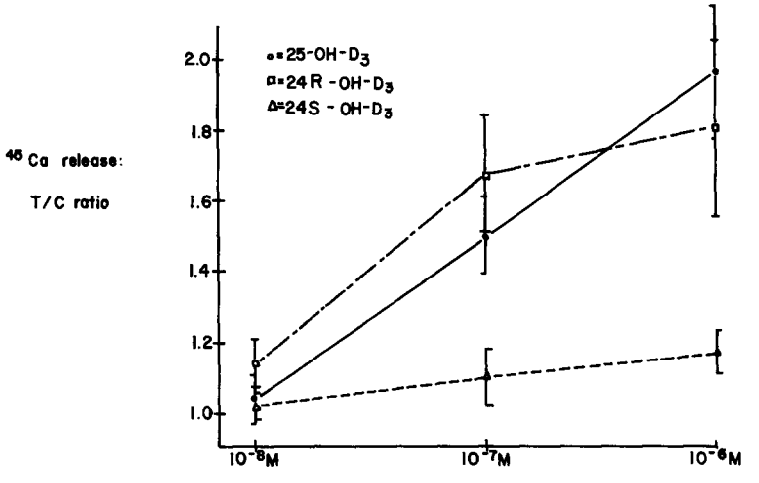

Figure 1. Effects of 24(S)-OH-D_3, 24(R)-OH-D_3 and 25-OH-D_3 on bone resorption in vitro. Fetal rat long bones were incubated for 48 hours with the vitamin D derivative. Values are expressed as the ratio of ^{45}Ca released from treated and control bones. Each point is the mean ± standard error of responses from 6 bone pairs. Significant differences: at 10^{-7} M 24(S)-OH-D_3 and 24(R)-OH-D_3, $p < .02$; 24(S)-OH-D_3 and 10^{-7} M 25-OH-D_3, $p < .02$; at 10^{-6} M 24(S)-OH-D_3 and 24(R)-OH-D_3, $p < .05$; 24(S)-OH-D_3 and 25-OH-D_3, $p < .01$; 10^{-6} M 24(S)-OH-D_3 and 10^{-7} M 24(R)-OH-D_3, $p < .05$; 10^{-6} M 24(S)-OH-D_3 and 10^{-7} M 25-OH-D_3, $p < .05$.

Methods

19-day fetal rat radii and ulnae prelabelled with [^{45}Ca]$CaCl_2$ were cultured in medium BGJ (Fitton-Jackson modification, Grand Island Biological Co.) with a modified salt solution (11). To reduce exchangeable calcium, the bones were first precultured without bicarbonate or albumin for 5-8 hours. Subsequently the bones were transferred to a bicarbonate-buffered medium containing 1 mg/ml bovine serum albumin and the vitamin D metabolites. The ^{45}Ca released into the culture medium during a 48-hour incubation was measured by liquid scintillation counting. Details of the method have been published previously (11,12). 24(R),25-$(OH)_2D_3$, 24(S),25-$(OH)_2D_3$, 24(R)-OH-D_3 and 24(S)-OH-D_3 were synthetic derivatives (1,4). 25-OH-D_3 was a gift of the Upjohn Company. Compounds were added in 95% ethanol. Final ethanol concentrations were less then 0.5%. Where appropriate, ethanol was added to control cultures. All of the compounds were tested in each experiment. Differences were tested for significance by a two-tailed Student "t" test.

Results

Figure 1 compares the responses of fetal rat bones to 24(S)-OH-D_3, 24(R)-OH-D_3 and 25-OH-D_3 over a 10^{-8} to 10^{-6} M concentration range. 25-OH-D_3 and 24(R)-OH-D_3 were approximately equal in potency; neither was effective at 10^{-8} M and the responses of the two were not significantly different at

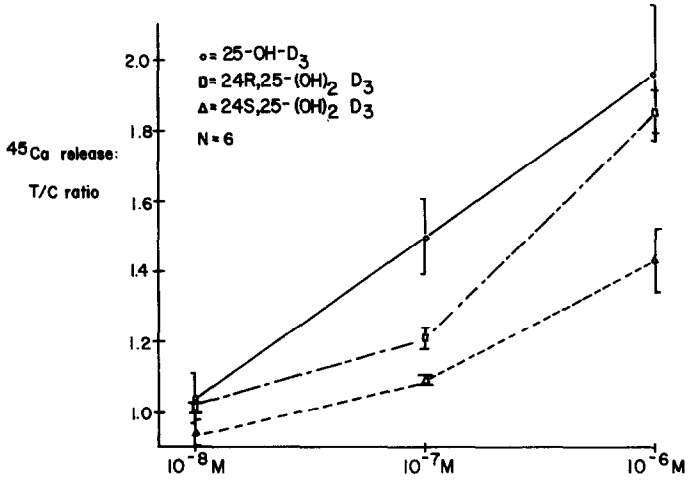

Figure 2. Effects of 24(S),25-$(OH)_2D_3$, 24(R),25-$(OH)_2D_3$ and 25-OH-D_3 on bone resorption in vitro. Fetal rat long bones were incubated for 48 hours with the vitamin D derivative. Values are expressed as the ratio of ^{45}Ca released from treated and control bones. Each point is the mean ± standard error of responses from 6 bone pairs. Significant differences: at 10^{-7} M 24(S),25-$(OH)_2D_3$ and 24(R),25-$(OH)_2D_3$ $p < .02$; 24(S),25-$(OH)_2D_3$ and 25-OH-D_3, $p < .01$; 24(R),25-$(OH)_2D_3$ and 25-OH-D_3, $p < .05$; at 10^{-6} M 24(S),25-$(OH)_2D_3$ and 24(R),25-$(OH)_2D_3$, $p < .01$; 24(S),25-$(OH)_2D_3$ and 25-OH-D_3, $p < .05$.

10^{-7} and 10^{-6} M. 24(S)-OH-D_3, in contrast, was inactive at both 10^{-8} and 10^{-7} M. A small response was detected at 10^{-6} M; however, it was significantly less than the responses to 25-OH-D_3 and 24(R)-OH-D_3 at 10^{-7} and 10^{-6} M. In the same experiment (Figure 2), 24(S),25-$(OH)_2D_3$ was significantly less active than its R isomer at all three concentrations tested. 24(R),25-$(OH)_2D_3$ was inactive at 10^{-8} M. At 10^{-7} M it was significantly less active than 25-OH-D_3. At 10^{-6}, the responses to 24(R),25-$(OH)_2D_3$ and 25-OH-D_3 were not significantly different. Since the studies in Figures 1 and 2 were carried out simultaneously, it is also possible to make cross comparisons. 24(R)-OH-D_3 was more active than 24(R),25-$(OH)_2D_3$ at 10^{-7} M ($p < .02$) and not significantly different at the other two concentrations. 24(S),25-$(OH)_2D_3$ was more active than 24(S)-OH-D_3 at 10^{-6} M ($p < .05$) and not significantly different at the two lower concentrations.

Discussion

The *in vitro* data obtained with the four new vitamin D derivatives correspond strikingly closely to the reported effects of these compounds on bone *in vivo* (2,4). When animals on a low calcium, vitamin D-deficient diet were given 650 pmoles of 24(S)-OH-D_3, the serum calcium was not detectably elevated two days later. The same dose of 24(S),25-$(OH)_2D_3$ produced a small but not significant increase under the same conditions. 24(R)-OH-D_3 and 24(R),25-OH-D_3 produced significant elevations in serum calcium and the responses were not statistically different from those produced by 25-OH-D_3, although the effect of 24(R),25-$(OH)_2D_3$ was slightly smaller. The good agreement between the *in vitro* and *in vivo* results suggests that the difference between the effectiveness of the enantiomers is a consequence of the responsiveness of the bone to the different compounds rather than to distribution factors altering the availability or duration of exposure of the bones to the derivatives.

A second order of complexity is brought into the interpretation of the results by the observation that the *in vivo* effects of the compounds are

abolished by bilateral nephrectomy. In studies of the effects of vitamin D derivatives the requirement for intact kidneys has been interpreted to mean that the compounds must be 1-hydroxylated before they can act (13-15). Thus, the possibilities arise that these compounds either have direct effects on bone which are not dependent upon 1-hydroxylation or that the fetal bone tissue contains 1-hydroxylase which acts upon these compounds during the continuous exposure in vitro. The latter possibility seems unlikely in view of the unpublished observations of Holick, Trummel, Raisz and DeLuca that 25-OH-D_3 is not 1-hydroxylated in vitro (cited in 6). Also, the mothers of the fetuses used for our culture studies were being fed a normal calcium diet containing vitamin D. Such animals are unlikely to have very active 1-hydroxylation, based upon earlier studies of 1,25$(OH)_2D_3$ production (3). Obviously, a definitive answer to this question awaits direct studies of the 1-hydroxylation of the 24 and 24,25 hydroxylated vitamin D_3 derivatives by the cultured fetal bones. Some basis exists for postulating that these compounds could exert their in vitro effects without prior conversion to 1-hydroxylated products. In vitamin D-replete animals, which require higher concentrations of 25-OH-D_3 for effects, similar dose-response curves for bone mobilization were obtained whether the animals were intact or nephrectomized (16). Also, there is evidence that pharmacological doses of vitamin D_3 are effective in nephrectomized, D-deficient animals (17). It would be useful to know what blood levels are achieved with these high doses and to compare them with the concentrations required to elicit bone resorption in the current studies. In addition, studies of the relative effectiveness of high doses of the S and R isomers of 24-OH-D_3 and 24,25-$(OH)_2D_3$ in nephrectomized animals could aid in the interpretation of the in vitro findings. The effectiveness of 24,25-$(OH)_2D_3$ in vitro raises the possibility that this compound may be a mediator in the actions of pharmacologica doses of vitamin D_3 in nephrectomized animals.

24(R),OH-D_3 was equal in activity to 25-OH-D_3 in vitro, whereas 24(S)-OH-D_3 was much less potent. These observations raise the possibility that the 24

hydroxyl group when in the R configuration can serve as a substitute for the 25 hydroxyl group. Since 25-OH-D_3 and 24(R)-OH-D_3 had equivalent bone resorbing activity, it was intriguing that the compound with both hydroxyl groups, 24(R),25-$(OH)_2D_3$ was less, rather than more, active than either 25-OH-D_3 or 24(R)-OH-D_3. Solubility factors, steric hindrance or an excess of hydrophilic groups in this region of the molecule are possible reasons for the decreased activity. Alternatively, the 24(R),25-$(OH)_2D_3$ could have other activity which opposes the bone resorbing effect. In vivo studies with biologically generated 24,25-$(OH)_2D_3$ (which has been shown to be 24(R),25-$(OH)_2D_3$ (2)) indicated that it did not antagonize the bone calcium mobilizing activity of other vitamin D metabolites (18). In contrast to the above results with the 24R-hydroxy compounds, 24(S),25-$(OH)_2D_3$ was more active than 24(S)-OH-D_3 in culture. These observations should prove valuable in future attempts to envisage the molecular structure of the vitamin D receptor.

Acknowledgments

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