THE ACTION OF TWO METABOLITES OF VITAMIN D_3; 25,26-DIHYDROXYCHOLECALCIFEROL ($25,26(OH)_2D_3$) AND 24,25-DIHYDROXYCHOLECALCIFEROL ($24,25(OH)_2D_3$) ON BONE RESORPTION

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1. Introduction

In addition to the well known active metabolites of vitamin D_3, 25-OHD_3 and $1,25(OH)_2D_3$, two other dihydroxy derivatives of cholecalciferol have been isolated [1,2] from biological sources and identified [1–3] as 24,25- and $25,26(OH)_2D_3$. Their chemical synthesis was described recently by one of us [4–7] and by others [8,9]. Unlike 25-OH-D_3 and 1,25 $(OH)_2D_3$, which act on gut and bone [10,11], the two other metabolites of cholecalciferol appeared to have a more selective activity. In vitamin D-deficient rats 24,25- and $25,26(OH)_2D_3$-induced a stimulation of intestinal calcium transport but not of bone calcium mobilization [9,12]. Under slightly different conditions, however, larger doses of both metabolites caused an increase in bone resorption [13].

This apparent contradiction led us to investigate the action of 24,25- and $25,26(OH)_2D_3$ on bone resorption using a mouse bone culture system [14] and to establish the log-dose response curve of doses up to 600 ng/ml in terms of calcium, phosphate and hydroxy-proline release.

2. Methods

$24,25(OH)_2D_3$: The metabolite was prepared by chemical synthesis as described elsewhere [4,6]. The compound is a mixture of diastereoisomeres 24 R and 24 S in approximately equal proportions.

$25,26(OH)_2D_3$: The compound was prepared by chemical synthesis as described before [5,7] and consisted of a mixture of diasteroisomeres 25 R and 25 S in approximately equal proportions.

Bone culture: The methods are those described previously [14]. The calvaria from 5- to 7-day old mice are cultured in BGJ_b medium supplemented with 15% horse serum. After a 24 h equilibration period at 37°C in an atmosphere of 5% CO_2 95% air, the medium is replaced by fresh medium, 2 ml/culture, which in the test vessels contain 25-OHD_3, 24,25 $(OH)_2D_3$ or $25,26(OH)_2D_3$. The vitamin D metabolites are added to the culture medium dissolved in spectroscopic ethanol (0.01 μl/2 ml medium). Each experiment contains a control, a high and low dose of 25-OHD_3 and at least 3 doses of $24,25(OH)_2D_3$ or 25,26 $(OH)_2D_3$. The response to the metabolites is calculated as the mean change in medium calcium (7–10 calvaria) from the 7–10 control calvaria.

3. Results

There is a log-linear response in bone resorption to concentrations of 25-OHD_3 between 100 and 500

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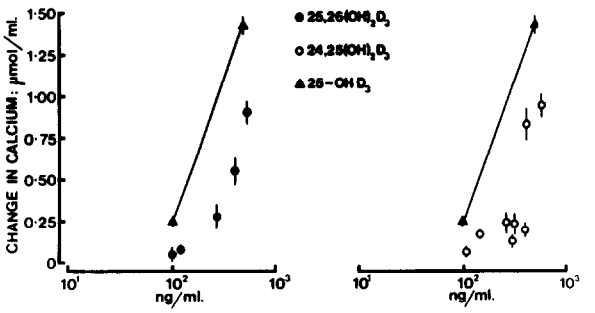

Fig.1. The log-dose response curves of mouse calvaria in tissue culture to 25-OHD_3 (▲), 25,26$(OH)_2D_3$ (●) and 24,25$(OH)_2D_3$ (○). The data have been combined from several experiments and are expressed as the mean change in medium calcium, µmoles/ml ± S.E.M., of the treated from the untreated calvaria.

ng/ml (fig.1). The metabolites 25,26$(OH)_2D_3$ and 24,25$(OH)_2D_3$ give a parallel response but at concentrations above 300 ng/ml (fig.1). A significant (p <0.001) increase, however, in bone resorption was given by 150 ng/ml of 24,25$(OH)_2D_3$ whereas no response was seen with 123 ng/ml of 25,26$(OH)_2D_3$. The change in medium calcium is associated with a corresponding change in medium phosphate (r = 0.99, p <0.001) and hydroxyproline (r = 0.85, p <0.001) and histology of treated calvaria showed an increase in resorption cavities as compared to control calvaria.

4. Discussion

The present results confirm that 25-OHD_3 at concentrations which are near to those found in human plasma, is very active in stimulating resorption in cultured bone [15,16]. The natural metabolites 24,25$(OH)_2D_3$ and 25,26$(OH)_2D_3$ present in plasma but at a lower concentration than 25-OHD_3, are also capable of resorbing bone. Both, however, are less potent than 25-OHD_3. Reynolds et al. [16] showed that 24,25$(OH)_2D_3$ did not resorb bone but concentrations higher than 50 ng/ml were not tested. The

Table 1
Effects of metabolites of vitamin D_3 on the release of calcium in the medium

Vit. D. metabolite	Concentration (ng/ml)	Change in calcium µmoles/ml (± 1 S.E.M.) Mean	± S.E.M.	n	p
24,25$(OH)_2D_3$	68	0.060	0.064	8	<0.5
	75	0.061	0.054	8	<0.5
	110	0.025	0.043	8	<0.5
	150	0.165	0.022	8	<0.001
	270	0.230	0.065	8	<0.01
	300	0.117	0.042	7	<0.05
	300	0.235	0.066	8	<0.05
	400	0.193	0.037	8	<0.01
	500	0.835	0.095	8	<0.001
	600	0.938	0.071	8	<0.001
25,26$(OH)_2D_3$	50	0.119	0.060	7	<0.1
	100	0.020	0.043	8	<0.5
	123	0.072	0.014	8	<0.1
	300	0.269	0.077	7	<0.01
	400	0.533	0.078	8	<0.001
	518	0.905	0.064	8	<0.001
25-OHD_3	100	0.237	0.030	39	<0.001
	500	1.445	0.054	40	<0.001

p The p values give the significance of the difference from the controls (Students' t - test).

hydroxyl group at the 25 position is important in the bone resorbing activity of vitamin D. So far we have found no vitamin D analogue with a 25 hydroxyl group which is unable to resorb cultured bone (personal data). It is clear, however, that hydroxylation at other sites can decrease or increase the bone resorbing activity.

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